The "Aquaculture Handbook – Fish Farming and Nutrition in Pakistan" was produced in conjunction with local Pakistani authors by the American Soybean Association's World Initiative for Soy in Human Health (ASA/WISHH). The handbook was produced as part of the U.S. Department of Agriculture (USDA)-funded "FEEDing Pakistan" project developed to improve capacity, productivity and quality in the Pakistani aquaculture sector through the active participation of public and private stakeholders. The project demonstrated improvements in fish growth and survival through the use of floating fish feed produced in Pakistan with U.S. soybean meal. Feed produced with high quality soy protein not only supports growth and healthy development of fish but also reduces costs and supports healthy environment initiatives.

The advances made under FEEDing Pakistan were due primarily to the integral participation and dedication of Pakistani fish farmers, academics, members of the private sector, and government officials. Participants' desire for increased knowledge led to the rapid adoption of new technologies and best management practices. The subsequent private investment of time and resources by both individuals and institutions created a framework for continued growth of the Pakistani aquaculture sector for years to come promising to ultimately improve food security in Pakistan.

Elizabeth Hare
Project Director, FEEDing Pakistan
ASA/WISHH
U.S.A.

R.S.N Janjua
Country Representative
ASA/WISHH
Pakistan

Supported By
USDA

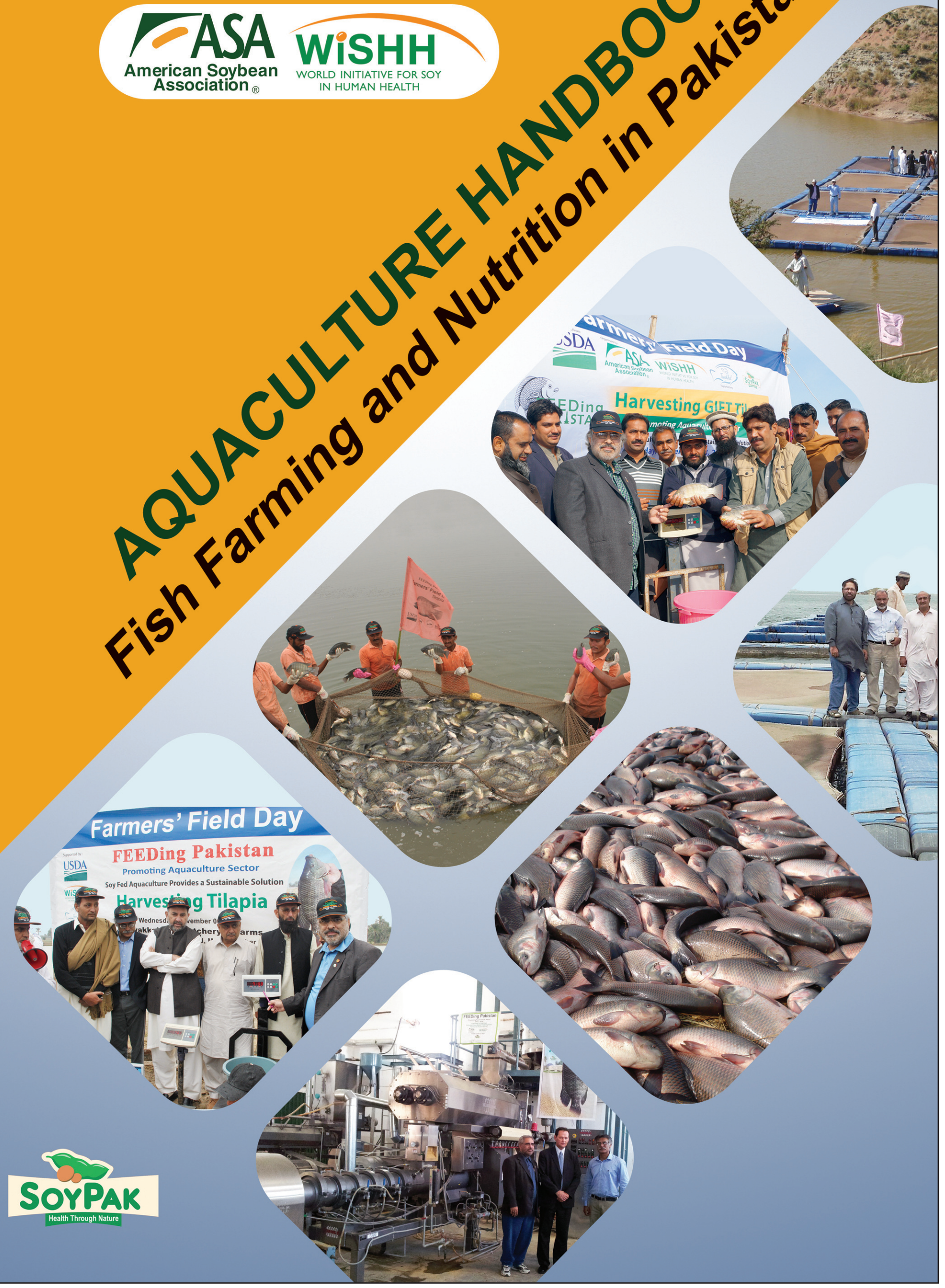
AQUACULTURE HANDBOOK
Fish Farming and Nutrition in Pakistan

PAKISTAN

FEEDing PAKISTAN
SOY FED AQUACULTURE PROVIDES A SUSTAINABLE SOLUTION

ASA American Soybean Association
WISHH WORLD INITIATIVE FOR SOY IN HUMAN HEALTH

AQUACULTURE HANDBOOK
Fish Farming and Nutrition in Pakistan



SOYPAK
Health Through Nature

FEEDing PAKISTAN

SOY FED AQUACULTURE PROVIDES A SUSTAINABLE SOLUTION

Aquaculture Handbook

Fish Farming and Nutrition in Pakistan

Lead Editor

Dr. Kevin Fitzsimmons, Ph.D.
Past President, World Aquaculture Society
Professor of Soil, Water and Environmental Science
Director for International Agriculture Programs
1140 E. South Campus Drive
Forbes 306, University of Arizona
Tucson, AZ 85719, U.S.A.

Co-editor

Dr. R.S.N. Janjua, Ph.D.
Country Representative
American Soybean Association
World Initiative for Soy in Human Health
Pakistan

Local Lead Author

Prof. Dr. Muhammad Ashraf, Ph.D.
Dean, Faculty of Fisheries and Wildlife
University of Veterinary and Animal Sciences
Pattoki Campus, Punjab – Pakistan

Supported By

USDA

ASA American Soybean Association

WISHH WORLD INITIATIVE FOR SOY IN HUMAN HEALTH

SOYPAK

Aquaculture Handbook - Fish Farming and Nutritionin Pakistan

First Printing: 2015, Pakistan

ISBN #
978-969-9564-52-4

Printed in Pakistan
58-3148-1-180-012015-1000

Design and Production:

SoyPak (ASA/WISHH)
4th Floor, Gulistan Chamber
Z-1-M, Shabbirabad
Karachi-75350
Tel: +92 21 3454 1721
Fax: +92 21 3454 1722
Email: info@soypak.org

Contents

Preface

Agriculture is a major contributor to Pakistan's economy with the livestock sector contributing up to 55% of the country's gross domestic product (GDP), and the Fishery Sector contributing close to 1%. The Fishery sector in Pakistan has not grown as rapidly as others in the last several years; however, given theincreased need for protein in the country, both the public and private sectors are committed to its development.

The American Soybean Association (ASA) has extensive experience in Pakistan working with stakeholders inthe poultry, livestock, dairy and food industries. ASA's efforts have been focused on knowledge dissemination and technology transfer through a variety of educational activities. Previous publications by the ASA in Pakistan include:"Manual of Feed Microscopy and Quality Control", "Handbook on Poultry Diseases", and the "Handbook of Poultry Nutrition".

The "Aquaculture Handbook – Fish Farming and Nutrition in Pakistan" was produced in conjunction with local Pakistani authors by the American Soybean Association's World Initiative for Soy in Human Health (ASA/WISHH). The handbook was produced as part of the U.S. Department of Agriculture (USDA)-funded "FEEDing Pakistan" project developed to improve capacity, productivity and quality in the Pakistani aquaculture sector through the active participation of public and private stakeholders. The project demonstrated improvements in fish growth and survival through the use of soy-based, floating fish feed produced in Pakistan with U.S. soybean meal. Feed produced with high quality soy protein not only supports growth and healthy development of fish but also reduces costs and supports healthy environment initiatives.

This handbook covers all facets of aquaculture from the initial construction of fish farms through farm management, feeding, fish disease, marketing, processing and the economics of tilapia production.

ASA/WISHH presents this book to stakeholders in the Pakistani Aquaculture Sector to serve asa source of continuous guidance as the sector develops.We hope that the Aquaculture Handbook will serve as a tool to improve the incomes of Pakistanifish farmersandfood security in Pakistan.

Jim Hershey
Executive Director
ASA/WISHH
St. Louis, MO, USA

Acknowledgments

Activities under the FEEDing Pakistan project have included the collaboration of a variety of stakeholders including: the Pakistan Fisheries Development Board, the University of Veterinary and Animal Sciences, Kansas State University (KSU), local fish farmers, Pakistani government representatives, and a variety of private sector Pakistani companies.

ASA/WISHHwould like to thank the following individuals for their work on this book as well as their dedication to the continued improvement of the Pakistani Aquaculture Sector: Dr. Kevin Fitzsimmons who served as lead editor, Dr. Muhammad Ashraf who served as lead author, Dr. Otto Gonzalez and Mr. Matt Stellbauer of the US. Department of Agriculture who provided continuous project support and edits, all co-authors, both federal and provincial fisheries departments, academia, fish farmers, theentrepreneurs behind Pakistan's first extruded feed mill, thedevelopers of Pakistan's first tilapia hatchery, and Dr. R.S.N. Janjua who serves asASA/WISHH's Country Representative in Pakistan without whom this handbook would not have been possible.

The advances made under FEEDing Pakistan are due primarily to the integral participation and dedication of Pakistani fish farmers, academics, members of the private sector, and government officials. The production of this handbook was only possible through their collaboration and contributions.

Jim Hershey
Executive Director
ASA/WISHH
St. Louis, MO, USA

CHAPTER – I

INTRODUCTION TO AQUACULTURE IN PAKISTAN

Author
Dr. Muhammad Naeem Khan
Professor
Department of Zoology, University of the Punjab,
Lahore-Pakistan
E-mail: naeem.zool@pu.edu.pk

Co-Author
Mr. Anser Mahmood Chatta
Deputy Director Fisheries & Principal
Fisheries Research & Training Institute, Manawan,
Lahore-Pakistan
E-mail: ansermchatta@yahoo.com

1- INTRODUCTION TO AQUACULTURE

Aquaculture, the fastest growing food-producing sector, now accounts for more than 50 percent of the world's food fish and is perceived as having the greatest potential to meet the growing demand for aquatic food. Aquaculture is increasingly emerging as one of the fastest growing and important economical agribusinesses, worldwide (FAO, 2014). The demand for fish is expected to exceed all available supplies in the near future owing to the revolutionary changes taking place in the dietary habits of people all over the world and to the medical community's promotion of fishery products as health food. Today the food basket of millions of people around the world is dependent on seafood's protein and other nutritional requirements. Fish and fishery products recorded the highest increase in price, both in domestic and export markets in recent years, compared to any other food item (Ninawe, 2009).

1. A. Definition

Aquaculture, also known as aqua-farming, is the farming or husbandry of aquatic organisms of economic or commercial value, such as: fish, crustaceans, mollusks and aquatic plants. Therefore aquaculture can be regarded as the aquatic counterpart of agriculture. Today aquaculture is agreed to be the art, science, and business of cultivating aquatic animals and plants in fresh or marine waters. However, its more technical definition is an industry that embraces the cultivation of aquatic organisms (whole or part of the life cycle) under controlled or semi-controlled systems for commercial, recreational or resource management purposes (Chaudhuri, 2009). Therefore, today the most widely accepted, short definition of aquaculture is the cultivation of any aquatic (freshwater and marine) species of plant or animal. Aquaculture involving cultivation of freshwater or saltwater populations under controlled conditions can be contrasted with commercial fishing, which is the harvesting of wild fish (Jadhav, 2009).

The huge biodiversity of organisms is also reflected in the choice of species for aquaculture. These aquaculture species can range from unicellular algae to giant seaweeds, while among animals, from microscopic protozoan to crustaceans and mollusks, such as shrimps, prawns, crabs, lobster, clams, oyster, scallops, mussels and fish species such as carps, salmon, trout, catfish, tilapia, flounder, striped bass, cod, eel, sturgeon, halibut and other freshwater and marine fish species. Fish aquaculture is differentiated from harvesting of natural fish resources by the interventions in the rearing process of the organism to enhance production. These interventions may include stocking, manuring, fertilization, feeding and protection from predators. Therefore, aquaculture is the man made attempt to improve the production of valuable aquatic organisms by labor and energy input, through scientific and technological manipulations to improve and maximize survival, growth and reproduction of these species. Through these manipulations, a maximum yield of cultivated animal or plant is obtained within a minimum period of time to obtain food for human consumption, sport fish, bait fish, ornamental fishes, pearls or seaweeds etc. (FAO, 2004).

Moreover, while the aquaculture farming implies the individual or corporate ownership of the cultivated stocks, the capture fisheries of the natural resources does not come under the definition of aquaculture as harvesting of fish or other organisms from wild or natural waters would not include ownership or any other intended human intervention to enhance production.

1. B. History of Aquaculture

Hunting and fishing are the oldest professions of mankind since man began to search for food (Jha, 2010). Adoption of farming arose as a natural evolution from fishing and gathering of fish. Aquaculture evolved repeatedly in different civilizations and remained multi-locational and isolated, having its own pattern at each location, until recent advancements in culture techniques, species specific standardized practices and technology transfer under modern globalization. Aquaculture practices can be traced back to the period 2000-1000 BC, which shows that aquaculture has a long history dating as far past as 4,000 years ago. Most of the literature on aquaculture refers to the historical past of fish culture in ancient Egypt, Asia and in central Europe (FAO, 1998).

Pictographic engravings on an ancient Egyptian tomb showing tilapia being fished out from a manmade fish pond reveal that Egyptians were probably the pioneers in the world of fish culture, as far back as 2500 BC. Chinese raised carp as food in freshwater ponds around 2000 BC. Undoubtedly, China was the cradle of carp culture, especially of *Cyprinus carpio*, however, due to absence of paper and printing, no written records of fish culture from that period are available (FAO, 1987). The first written record of fish culture techniques is attributed to a Chinese politician Fan Lei, who became a fish culturist and is often described as the 'Father of Aquaculture'. He wrote his book, "*Classic of Fish Culture"* in 475 BC, describing pond construction, propagation methods and growth characteristics of common carp. It is presumed that commercial fish culture existed in China at his time, as Lei noted fish ponds as the source of wealth (Ling, 1997). A ban on culture of common carp by Emperor 'Lee' of the Tang Dynasty, in 6th century AD, led to the culture of other so-called Chinese carps and thus the polyculture aquaculture system evolved.

Chinese carried this knowledge of traditional carp culture to Indonesia, Malaysia, Thailand, Cambodia, Taiwan, Vietnam, etc., as they emigrated. In the Philippines, fish culture in brackish water ponds has been in practice for centuries. By 200 AD aquaculture in Japan and Korea had spread, perhaps influenced by China, using goldfish and carp for culture. Aquaculture continued to flourish and now many of the aquatic fish and shellfish species in Japan and Korea, especially of high economic value, are cultured for trade. Use of efficient, intensive culture technology is a distinctive feature of Korean and Japanese aquaculture (Jhingran, 1987).

In Central Europe, aquaculture began in medieval times, mainly in churches, rulers' palaces and monasteries. Common carp and trout culture developed in water bodies with these places. Aristotle mentioned oyster culture in Greece, while Pliny has given details of similar culture in Rome. In North America, fish culture developed very late in the 19th century with special emphasis on sport fishing with stocking of trout in cold waters and black bass in warm waters.

In Africa, except for tilapia culture in ancient Egypt, the origin of aquaculture is quite recent. Attempts were made to spread fish culture from Europe to Africa in the 1920's and 30's but didn't succeed due to nomadic nature of most of the African communities. After World War II, aquaculture has developed in Africa. Tilapia is the prize fish there, and since the 1930's has been extensively transplanted worldwide in almost all tropical countries.

In the Indian subcontinent, fish culture also has a long history dating back 3000 years. Fish remains with cut marks, the indication of their use as food; have been obtained from excavations of old Indus Valley civilizations of Mohenjo-Daro and Harappa (2500 BC-1500 BC) (Jha, 2010). King Somesvara had composed a book in 1127 AD on sport fishes. The traditional fish culture practice in ponds is known to exist in eastern India, for hundreds of years (Jadhav, 2009). Polyculture of native Indian major carps flourished in different parts of the sub-continent including present day Pakistan, till the start of composite fish culture of Indian and Chinese carps in the 1980's. Rapid growth and development of aquaculture during the last 4-5 decades has been attributed mainly to the development of artificial fish breeding (e.g. hypophysation), development of egg incubation & hatchery rearing techniques, development and availability of commercial fish feed using feed ingredients of agricultural and animal by-products, including availability of extruded pelleted floating feeds and development of fish transport amenities over the long distances.

1. C. Aquaculture: A global perspective

Although traditional fish farming was a part of rural life in some parts of the world, today's aquaculture has a significant role in socio-economic development, uplift of rural communities, foreign exchange earnings and management of natural resources. Fisheries and aquaculture provides livelihood to about 55 million people engaged in the sector, including 7 million sporadic fishers and fish farmers. Besides production, it also provides plentiful jobs to the people involved in ancillary activities like harvesting, processing, packing and marketing, fish processing equipment, boat construction and manufacturing of fishing nets and gears, etc. (FAO, 2012).

Aquaculture presently is the fastest growing food producing sector among and accounts for more than 50 percent of the world food fish production. Its contribution to global seafood supplies in comparison to capture fisheries is increasing steadily for last few decades with average growth rate 8 percent per year from 1961 to 2013 while world population was increasing 1.7 percent per year. The overall per capita annual seafood consumption has also increased from 9.9 kg to 18.8 kg from 1960s to 2011, being lowest in Africa (9.1 kg) while Asia on the whole accounted for 20.7 kg per capita consumption per annum (FAO, 2012).

Capture fisheries is almost stagnant at about 90 million tonnes for the last three decades. In global aquaculture production of freshwater fishes dominate with 33.7 million tonnes production, followed by molluscs (23.6 million tonnes), crustaceans (5.7 million tonnes) and other aquatic animals (5.8 million tonnes) FAO (2012).

In last three decades, from 1980 to 2010, aquaculture has expanded virtually 12 times, with an average annual growth rate of 8.8 percent. In 2010, the world aquaculture production (including aquatic plant and non-food products) was 79 million tonnes amounting to US$ 125 billion. However keeping in view the projected world population growth over next two decades, it is estimated that in 2030, to maintain current per capita consumption, an additional 40 million tonnes of seafood will be required. Presently, 190 countries are raising around 600 aquatic species worldwide in captivity for production in farming systems of diverse input concentrations and technologies. Asia accounted for 89 percent of global aquaculture production, dominated by China which accounts for more than 60 percent world aquaculture production. China has been the main contributor for increase in world per capita fish consumption, as people migrate to the coasts for work and the overall standard of living increases and more people can afford seafood. India is the second largest aquaculture producing country while other major contributing countries are Viet Nam, Indonesia, Bangladesh, Thailand, Myanmar, Philippines and Japan (FAO, 2012).

Aquaculture is highly diverse and fragmented, ranging from small-scale fish ponds to cooperatives and international companies with annual turnover exceeding US$ billion. In Asia, freshwater aquaculture is predominant and is dominated by finfishes, mainly carps and tilapias. In North America catfish, trout and salmon aquaculture are the biggest sectors, but have grown only slightly since 2006. While in South America, particularly in Peru, Colombia, Ecuador and Brazil, aquaculture is continuously progressing and increasing production amounts, especially for tilapia and shrimp. In Europe, brackish and marine aquaculture of Atlantic salmon, sea bass, sea bream, tunas, mussels and oysters are dominant and in recent years have demonstrated slow overall growth. Africa has increased its contribution in world aquaculture production with the development of freshwater finfish culture since 2000, while Oceana accounts for only a small share of global aquaculture production consisting mainly of marine finfish and mollusks (FAO, 2014).

Inter-regional transplantation of aquaculture species has also made a significant contribution to the Blue Revolution. The main transplanted species worldwide are common carp, rainbow trout, tilapias and Chinese carps (grass, bighead and silver carp). The increasing domestication of various fishes, crustaceans, molluscs and even seaweeds has vastly increased yields and profitability. The life cycles of many species has been "closed", meaning that multiple generations can be reared in captivity. Traditional selective breeding has provided huge gains in productivity. Many species have now had their genetic sequences described in the literature. Using new genetic markers and vastly increased knowledge of genomics and proteomics, an additional set of gains in selective breeding is expected. The use of trans-species genetic engineering has not reached commercialization yet, and will probably provide little if any productivity gains before 2020.

Freshwater fish production is dominated by carps, tilapia and *Pangasius* and other catfishes while coastal aquaculture production principally comprises penaeid shrimps, oysters, scallops, mussels and Atlantic salmon as leading farmed marine species (Bostock *et al*., 2010). Freshwater aquaculture production is dominated by pond farming where carps and other lower trophic level fish species are cultured under semi-intensive pond culture systems using organic and inorganic fertilizers and low-protein, low-cost supplementary feeds. In contrast salmons and trout are raised in concrete raceways and tanks having 2-5 times higher stocking densities when compared with semi-intensive ponds and are fed with complete diets. Cage culture, both in freshwater and brackish water has also been flourishing in many countries. There is a rapid evolution towards more intensive farming in ponds as farmers add aeration devices and more complete diets. Careful pond water quality monitoring and control is a pre-requisite for success.

The tremendous global aquaculture explosion during the last three decades has raised some serious environmental questions as to the sustainability of aquaculture industry. Certain environmental groups have raised questions regarding introduction of exotic species, genetic modification or organisms, dilution of genetic integrity of natural stocks by hatchery raised, escaped farmed fish, harvesting of pelagic fishes for preparation of fish meal, use of antibiotics and chemicals during fish farming, release of fecal wastes & effluents leading to algal blooms and excessive extraction of ground water for pond farming. This has led to development of stricter aquaculture laws and regulations in many countries and to Best Management Practices promoted by industry leaders and non-governmental organizations (NGO's). The intensive aquaculture systems require high protein diets containing fish meal and fish oils. Small, low priced "trash" fish are directly used to feed some marine species, which is not only ecological but is a social issue for some people. Similarly, a lot of low quality fish is caught to use as fish meal to prepare high protein fish and shrimp diets. Development of soybean meal based fish feeds has replaced fish meal as an excellent source of protein. The amino acid profile of soybean meal is similar to fish meal and has proven to be the best substitute so far. Directed breeding programs have brought some varieties even closer to fish meal further increasing its beneficial use. Several other protein sources including poultry by-product meal, feather meal, meat and blood meals, sunflower, cotton, sesame, and camelina seed meals, corn protein concentrates, dried distillers grains, yeast based ingredients and ethanol by-products are also now commonly used as fish meal replacements.

1. D. Status of Aquaculture in Pakistan

1. D.1. *General Overview*

Pakistan has vast fresh, brackish and marine water resources with a 1046 km long coastline. Due to its location as the drainage basin for the western Himalayas, Pakistan has substantial areas of inland waters. The region between 33° N and 20° N consists of a network of rivers, canals, reservoirs, lakes, waterlogged areas and village ponds, etc. with a total area of about 8.6 million hectares.

Annually, 0.6 million tonnes fish is produced, in which marine and inland share is 63 percent and 37 percent respectively; aquaculture contributes 15 percent of the total fish production. Fifty percent of the total produced is consumed domestically, 20 percent is exported while remaining 30 percent is used as fish meal. Per capita fish consumption in Pakistan is 2 kg, which is the lowest in the world of any country with a seacoast. The fisheries sector contributes one percent to the country's GDP (FAO, 2013) and three percent to the agriculture GDP; provides livelihood for 400,000 fishermen while another 600,000 people are involved in ancillary activities.

Fisheries and aquaculture in Pakistan is basically a provincial responsibility. At the central level, fisheries are the responsibility of the office of the Fisheries Development Commissioner (FDC), attached to the Ministry of Ports & Shipping. FDC is responsible for policy, planning and coordination with provincial fisheries departments and other national and international agencies. Freshwater carps farming is the major aquaculture activity in the provinces of Punjab and Sindh, while in Khyber Pakhtunkhwa (KPK) Province both carps and trout are cultured. In Balochistan Province, with the efforts of provincial government, freshwater carps farming at a small scale has recently been started in the private sector.

In Azad Jammu & Kashmir (AJK) and Gilgit Baltistan Territory, culture of some cold water and semi-cold water fish species, at a limited scale is in practice. Pakistan is endowed with a rich freshwater fish fauna, comprising above 200 species, but only seven warm water and two cold water fish species are commercially cultivated in the country. Presently no coastal or marine aquaculture exists in Pakistan.

Knowledge of fish in Indian Sub-continent is very old. During the excavations of old civilizations of Harappa and Mohenjo-Daro

(2500 BC-1500 BC), some indications of fish used as food have been found (Jha, 2010). The early experiments on breeding of indigenous major carps (Rohu and Mori), in the Indian sub-continent were conducted in the 1930s, by Dr. Hamid Khan Bhatti, at Chhenawan Head works, District Gujranwala. However aquaculture practices in Pakistan were started in recent past. In 1970's there were a few fish farms with very low production per unit area. In the beginning, aquaculture practices were few and growth rate of aquaculture was slow due to inadequate supply of seed of required fish species and lack of proper culture techniques. At that time, as a common practice, fish seed of different species was collected from natural fish breeding grounds of rivers and "dhunds" after monsoon floods, sorted out and supplied to fish farmers for their small fish ponds.

The success in artificial breeding of indigenous major carps was a major breakthrough achieved in late 1970's, to move the sector forward. The introduction of some fast growing Chinese carps (Grass carp and Silver carp) in the late 1980's, and the improvement in culture techniques, further helped aquaculture to grow and prosper at a faster rate. For the last two decades, aquaculture in Pakistan is progressing with annual growth rate 10-15%. The stepwise development and transfer of technology of artificial propagation of carps in Pakistan can be outlined:

- Up to 1970 — Collection of seed from nature after monsoon floods
- 1970-75 — Introduction of induced spawning technique using pituitary gland (PG) extract
- 1975-85 — Use of Chinese breeding technology

-	1985 onward	Use of glass jar hatchery techniques & circular spawning tanks for egg incubation
-	1992 to update	Use of synthetic hormones like Ovaprim for artificial breeding

1. D.2 *Cultured Fish Species*

Up to the 1980's, most of the Pakistani fish farmers stocked their ponds only with indigenous major carps (Rohu, Mori and Catla). Then some fast growing Chinese carps (grass carp, silver carp, common carp and big head carp) were introduced for mixed culture under modern polyculture system to increase fish yield per acre. Today earthen pond polyculture of indigenous major carps and Chinese carps with 4-5 species, in different combinations and ratios has become very popular amongst the fish farmers. In 2014 the following fish species are being cultured in Pakistan (Table 1.1).

Mahseer, a semi-cold water game fish, Sol (a local snakehead) & Singhari (a local catfish) have tremendous potential as new aquaculture species in Pakistan. Efforts are being made to artificially breed these fish species to diversify species selection in pond culture system. Similarly some trials have been conducted on the culture of shrimp/ prawn in Sind and Punjab province but without any significant success.

Table 1.1. Fish species being aquacultured in Pakistan

S. No.	Common Name	Scientific Name	Level
1	Rohu	*Labeo rohita*	Commercial
2	Mori	*Cirrhinus mrigala*	Commercial
3	Thaila	*Catla catla*	Commercial
4	Common Carp	*Cyprinus carpio*	Commercial
5	Silver Carp	*Hypophthalmichthys molitrix*	Commercial
6	Grass Carp	*Ctenopharyngodon idellus*	Commercial
7	Bighead Carp	*Aristichthys nobilis*	Commercial
8	Rainbow Trout	*Oncorhynchus mykiss*	Commercial
9	Tilapia	*Oreochromis niloticus*	Commercial
10	Mahseer	*Tor macrolepis*	Pilot
11	Sol	*Chana marolius*	Pilot
12	Singhari	*Mistus singhala*	Pilot
13	Pangasius	*Pangasius pangasius*	Commercial
14	Channel catfish	*Ictalurus punctatus*	Pilot
15	Shrimp	*Macrobracium spp:*	Pilot

1. D.3. *Fish Production Systems*

Pakistan is practicing varied aquaculture systems including, extensive fish culture in lakes and reservoirs, cage & pen culture in lakes and reservoirs, raceway & tank culture and the most popular, the earthen pond fish culture. Rural pond culture in Pakistan has moved from subsistence to small and medium scale commercial culture of carps in extensive and semi-intensive level. While the extensive culture is done in lakes and dams of Sind and Punjab Provinces, the pen culture and cage culture of Tilapia and carps has been increasing slowly. Similarly, the raceway/ tank culture is in practice for cold water trout and semi-cold water Mahseer species, in Khyber Pakhtunkhaw (KPK) and northern Punjab.

The main fish culture system, widely used in Pakistan, is semi-intensive pond polyculture system. In these ponds, fish species of different feeding habits and trophic levels i.e. surface; column and bottom feeders are stocked in a specific ratio to avoid feed overlapping. Hatchery produced fish seed of carps i.e. Rohu, Mori, Thaila, Silver carp and Grass carp, are generally stocked, with stocking density of 825 per acre, allowing 10 percent mortality. The existing recommended ratio and number of fish per acre is given in the Table-1.2, below:

Table 1.2. Per acre stocking ratio of fish seed

Fish species	Number of fish / acre
Rohu	310
Mori	103
Thaila	103
Silver carp	206
Grass carp	103

However, stocking density, stocking ratio and stocking combinations are adjusted, keeping in view the nature of ponds, market demand of fish species, use of fish feed, primary productivity of the pond and the level of technology & intensification of aquaculture system. In semi-intensive pond polyculture systems, supplementary feed, composed of local and relatively cheaper feed ingredients are normally used to get marketable size with faster growth. The most common feed ingredients used in Punjab are rice bran or polishing, maize gluten and oilseed cakes. Sometimes fish meal is also added @ 5-10 percent, to improve the nutritional value of feed.

Recently international aid and investments in fish feed manufacturing has brightened the prospects of aquaculture growth in Pakistan (Oryza Organics, 2013). The availability of commercial floating fish feed prepared on extruders with varied particle size and protein composition levels is expected to boost and revolutionize the aquaculture sector in Pakistan, as one of the most important bottleneck in terms of fish feed has been removed. It is expected that the present average yield of 1,000-1,200Kg fish/acre/annum can be increased to 7,000-8,000Kg fish/acre/annum, ushering a new era of modern sustainable industrial aquaculture.

1. E. Should I invest in Aquaculture?

The aquaculture industry with an impressive and unprecedented present growth rate of 10-15 % compared to agriculture, livestock, poultry and other food producing sectors has grabbed the attention of investors, multinational companies, banks and corporate bodies and progressive fish culturists globally. In Pakistan, it has opened up avenues of provision of subsistence, employment and job opportunities to the rural communities for integrated rural development. Today many countries of the world, particularly the lower and middle income countries of Asia, depend on aquaculture not only for their food but also for earning foreign exchange. Similarly millions of people around these countries depend highly on aquaculture for their survival and subsistence. Keeping in mind the need for additional food, especially protein, demands for the growing population of the world as a whole and the developing countries in particular, it is important to promote sustainable aquaculture practices.

The global decline in capture fishery has further highlighted the importance of fish production from alternate sources of aquaculture. Today, aquaculture is a most suitable agribusiness for investment due to its broad choice of species diversity, sustainability, consistency, ever increasing demand, potential for better rate of return and lesser risks compared to other farming systems, like poultry and livestock. Importantly, fish is the only cash crop in Pakistan sold on net cash, while other crops like wheat, rice and other cereal grains are traditionally sold on loan basis to the creditors. Aquaculture has the potential to bring into use the waste and marginal agriculture lands, which are otherwise unfit for agricultural crops.

It is expected that R&D in aquaculture, transfer of technology, aquaculture legislation, export marketing and quality control measures including adoption of FAO Code of Conduct for Responsible Aquaculture through Good Management Practices (GMPs) would further help to boost the trust and confidence of Pakistani investors in the aquaculture sector.

References

Bostok, J., B. McAndrew, R. Richards, K. Jauncey, T. Telfer, K. Lorenzen, D. Little, L. Ross, N. Handisyde, I. Gatward and R. Corner, 2010. Aquaculture: global status and trends. Phil. Trans. R. Soc. B. (365):2897-2912.

Chaudhuri, A. B. 2009. Aquaculture, Limnology and Ichthyology. Daya Publishing House Delhi. 3 pp.

FAO, 1988. History of Aquaculture.

FAO, 2014. The State of World Fisheries and Aquaculture.

FAO. 1998. Report of the Technical Consultation on the Feasibility of Developing Non-Discriminatory Technical Guidelines for Eco-Labelling of Products from Marine Capture Fisheries. Rome, Italy, 21–23 October 1998. FAO Fisheries Report No. 594. Rome. 29 pp.

FAO. 2010. Report of the Expert Consultation on the Development of Guidelines for the Eco-labelling of Fish and Fishery Products from Inland Capture Fisheries. Rome, 25–27 May 2010. FAO Fisheries and Aquaculture Report No. 943. Rome.37 pp.

Jadhav, U., 2009. Aquaculture technology and environment. PIH Learning Private Limited, New Delhi, pp. 19-20.

Jha, K. K., 2010. Aquaculture. Daya Publishing House Delhi, pp. 1-4.

Jhingran, V. G.,1987. Introduction to Aquaculture. Based on lectures presented at African Regional Aquaculture Centre for Senior Aquaculturist Course, FAO.

Ling, S.W., 1997. Aquaculture in Southeast Asia: A historical overview, Washington Sea Grant Publication (Univ. of Washington Press), pp. 94-96.

Ninawe, A. S., 2009. Aquaculture technology and environment (Forward). PHI Learning Private Limited, New Delhi.

Oryza Organics, 2013. Innovating in feeds, Lahore, www.OryzaOrganics.com

CHAPTER - II

CONSTRUCTION AND MANAGEMENT OF AQUACULTURE FACILITY

Author
Professor Dr. Naeem Tariq Narejo
Chairman
Department of Fresh Water Biology & Fisheries
University of Sindh, Jamshoro – Pakistan
E-mail: dr_ntnarejo46@yahoo.com

Co-Author
Ms. Fozia Nazeer
Ph.D. Scholar
Department of Fresh Water Biology & Fisheries
University of Sindh, Jamshoro – Pakistan

1. INTRODUCTION

Fish culture in Asia is several thousand years old. The pioneers in fish culture are the Chinese who practiced it as far back as 475 B.C (Dill and Pillay, 1968). Even today, most of the principles of fish farming originated from Chinese literature. In Pakistan, the cultivation of fish is of recent origin. Serious attention has been given only a decade back to develop it on scientific lines. It is necessary to provide fish protein to the ever increasing population of Pakistan. Fish is a nutritious first class food. It contains protein in a palatable, digestible, and nutritious form, thus providing nutrients for growth and energy, as well as rejuvenation of worn out tissues. Additionally, it contains vitamins A and D, deficiency of which may lead to ulceration of the eye, night blindness, malnutrition, and increased susceptibility to diseases. Pakistan is blessed with vast natural resources that could be applied to aquaculture (Narejo, 2006). Fish farming remains an under-developed sector due to the lack of institutional facilities, shortage of fish seed of culture species, and lack of basic knowledge of industry practices. This handbook is an attempt to solve the basic problem of non-availability of technical information for the successful operation of commercial fish farming.

2. SELECTION OF SITE

One of the most important parts of planning a fish farm is finding a suitable place for the pond, because the success and profitability of a fish pond depends primarily on the selection of the site. The following factors should be taken into consideration while selecting a site for a fish pond.

ACCESSIBILITY OF POND SITE

A site away from roads should be avoided as transportations of fish seed, marketable fish, and farm machinery equipment would be difficult. Access to the pond at all times is very essential. Transportation costs can increase rapidly if materials are manually carried over long distances.

Availability of labor, energy or electricity, fish seed, fish feed ingredients, fertilizers, and extension services are very important factors. The pond should not be in flood affected areas or near previously polluted areas. Generally eroded areas are not suitable as flood events are likely to reoccur. As much as it is possible, a site in a high-density forest or with a high concentration of buildings should not be chosen because solar radiation and wind will be affected. Electricity poles should not stand in the water (Pillay, 1990). The prospective pond culturist should be familiar with the likelihood of poaching in this region, and plan accordingly.

There are three main factors that work together to make a good site for a fish pond.

a. Topography
b. Soil
c. Water

2.1 Topography

Topography refers to the "lay of the land" or the changes in the surface elevations of the ground, whether flat, rolling or sloping, undulating, or hilly. A crucial factor in pond construction is proper drainage of the pond, which is dependent on the land topography and determines the cost of pond construction. The ideal site for a fish pond is a flat area with a gentle slope (0.5-1.0%). Land with a greater than 5% slope is generally not suitable because of high cost of construction, siltation, and erosion problems (Martyshev 1973).

2.2 Soil

Generally the most suitable soil for a fish pond should contain a higher percentage (20-50%) of clay providing high water holding capacity. Sandy or sandy loam soils are not suitable because rate of seepage is higher. Furthermore, sandy banks will erode quickly and require frequent repair and maintenance. Fish farming is possible in sandy soil but it requires more management and involves more investment in soil amendments or purchase of plastic liners. It is always recommended that the soil composition of the proposed site be obtained through the soil Conservation Department, or any other appropriate agency, before the finalization of a site for pond construction (Martyshev, 1973).

A simple way to estimate the water retention soil at the field level is by squeezing a moist chunk of soil into a tight ball or rolling into a ribbon or cylinder. If it does not crumble after some handling or squeezing, it may be regarded as satisfactory.

2.3 Water

Water for fish farms can be drawn from many sources; watershed runoff, rivers, creeks, impoundments, small dams, lakes, irrigation canals, and underground (bore water). The type, size, location, and topography of a farm will determine the best or most practical source of water. Abundant supply of clean water is essential for commercial fish farming at a low cost. Therefore, it is desirable to construct a pond near the water source with guaranteed water supply throughout the year. Generally, canal and tube well water are considered good for fish farming. If the source of supply is from a tube well, it is desirable that water should flow in open air channels to absorb oxygen from the air as groundwater is often low in dissolved oxygen but high in toxic dissolved gases. Ground and surface water quality is affected by physical, chemical, and biological parameters. Of special concern are the by-products and wastes resulting from urbanization (fecal wastes, heavy metals and high biological oxygen demand) and agricultural pollutants such as pesticides, herbicides and fertilizers. Acidity and alkalinity of the water are important factors to be considered. Water ranging in pH from 6.5 to 9.0 is generally considered as most suitable for fish production. Excessively low or high pH beyond this range can cause lowered fish yield. Water with low pH or rapid swings in pH may be treated with lime to increase alkalinity. Pond water with low alkalinity (less than 20 mg/L) should be treated with agricultural lime to increase alkalinity to roughly 50 mg/L (Hall, 2002).

CLIMATE

Climatic conditions of the project area are also very important .The following elements concern fish farming operations: rainfall, temperature, and wind velocity. These factors greatly affect fish production directly or indirectly. Full climatic information should be obtained if a fish farming business is to thrive.

VEGETATION

The type and density of vegetation is also an important criterion in fishpond site selection. Avoid tall trees or densely populated small trees that block the sunlight. There is a direct relationship between vegetation and the amount and cost of pond construction work. In general, any heavily forested area will be too expensive to clear and will lead to eventual pond leakage as root systems decay (Hall 2002).

Areas with mangrove trees with dense rooting systems like *Rhizophora*, *Nypa* and *Avicennia* are the most difficult to excavate and frequently are protected ecosystems that will be illegal to clear. Mangrove areas also contain acidic soils that will make pond water management difficult in the future.

SECURITY

The site must be maintained to keep thieves from harvesting the fish. The pond water should not be available for domestic uses or access to cattle. Hoofs of cattle will damage pond banks and pond bottoms.

3. FISH CULTURE

There are three types of fish culture

a) Pond culture

b) Cage culture
c) Raceway culture

3.1 Fish Pond

A pond is a water impoundment used for the production of fish. These are usually smaller than lakes that are made by constructing a dam or dikes. These structures can be built on level lands, gently sloping lands, or sloping uplands valleys where soil construction, water resources and physical characteristics of the site are suitable. Primarily, two types of ponds are constructed for fish culture in Pakistan.

a) Watershed Pond

These are also called embankment ponds or barrage ponds. These ponds are created by building an embankment, dam, or dike or similar above–ground structure across a stream, water course or valley. The slope of the land plays a very important role in planning such ponds. A good site usually is one where a dam can be built across a narrow section of a valley. The sides have a moderate slope and there is wider section along its length allowing an area to be flooded to a depth of two to four meters. For ponds where surface run off is the main source of water, the contributing drainage area, i.e. watershed, must be large enough to maintain desired water levels. The quantity of water that can be expected to be stored from a given watershed depends upon individual and annual rainfall events and is affected by factors such as physical characteristics of the watershed. This is the reason why the appropriate size of a pond drainage area required for each acreage foot of storage in a dam pond varies from place to place. With the amount of rainfall we receive in the mountainous regions of the Punjab, approximately 15-25 acres of watershed area is required to fill a one-acre pond which is 2 meters deep. If the pond is to be filled mostly by streams, it is necessary to collect the information on the year round availability of the quantity of water before determining the final size of the proposed pond. The depth of dam ponds should be two to four meters with a minimum depth of two meters (Huet, 1994).

The foundation under a dam must ensure stable support for the structure and provide the necessary resistance to the passage of water. For this purpose the foundation condition under the proposed dam site should be thoroughly investigated by making soil borings. Good foundation materials are those that provide both stability and imperviousness such as a mixture of gravel, sand, clay, and sandy clay. The fill materials for building a dam should be available close to the site. The best fill material contains particles ranging from smaller gravel or coarse sand to fine sand and at least 20% by weight of clay particles. The required top width of a dam is up to three meters. in height. As the height of the dam increases, the top width also increases. The dam side slopes are dependent on the type of fill material used; the most common slope recommendation is 3:1. Another important consideration while building dams is to keep providing extra vertical capacity. Free board is the additional height of the dam provided as a safety factor to prevent over topping of the dam by wave action or other causes. It is the vertical distance from the pond surface when the spillway is discharging, to the top of dam after settlement (Huet, 1994).

A well-designed pond has three outlets, the mechanical spillway which carries the normal expected out flow from the pond, the emergency spillway to pass excessive storm run-off water when the flow exceeds the capacity of the mechanical spillway, and a drain system for times when the pond must be drained. After planning is completed, estimate the amount of earth required to build the dam carefully, this helps in estimating the cost to build. You must also confirm with authorities that your design is adequate, that you have water rights, and register the structure.

b) Excavated Ponds

These ponds are also called cut and fill or contour ponds. They are simple to build and are formed by digging soil from an area to form a pit or hole in the ground. The wall marks out the contour of a pond along one or up to four sides. The dike is aimed at retaining the water. The dike has four important features. The top or crown must be at least 1m or more, if it is to be used as a road. The height is equal to the depth of the water required plus 0.25m. The base width should be 2.5-5 times the wall height depending on soil characteristics and the slope 3:1 to 2:1. The pond size depends on whether the pond will be used for breeding, nursery, or production purposes. The production pond varying in size from one acre to four acres can be efficiently managed, however ponds as small as $200m^2$ can also be used for raising fish. Nearly any pond shape can be formed, but rectangular shapes are most common because they are easier to excavate and harvest.

It is best to build ponds close to a good supply of water such as a river canal, an existing borehole well, lake, or reservoir. In most of the plains of Pakistan, excavated ponds which are semi-above the grounds would be most suitable because of the ease with which they can be constructed, their compactness, and low maintenance requirements. Such ponds are formed by excavating about half a meter of soil and using the soil in building dikes (Meehan, 2002). The soil provided is enough to raise the dike 1.2 meters above the ground to have two meter depth. There are two kinds of excavated ponds, one that is filled with surface run-off, and one that is fed by sub-soil water. Some ponds can be designed to receive water from both surface run-off and wells. The excavated ponds which are filled with well water are normally located in flat or nearly flat lands and used for field crop irrigation. Ponds which are filled with surface run-off can be most satisfactorily built in comparatively flat areas with well drained terrain and again can be used for irrigation.

3.1.0 Design

Farm ponds can be designed either in series (linked one after another) or in parallel. Wind plays a role in fishpond design. Strong wind generates waves and waves breaking on the dikes will erode the sides of the dikes. To minimize this, position the longer pond dimensions (longer pond axis) parallel to the direction of the main prevailing wind direction so that the wave action would be on shorter length of dikes and erosion would be less.

3.1.1 Pond Preparation for Stocking

In a newly built pond, before stocking of fish seed, a layer of lime should be applied on the bottom. Agricultural lime (crushed limestone) is the safest to use. Hydrated lime is usually the least expensive, as it is more concentrated. Care should be taken if quick lime is used, as it can burn the skin and can also be harmful if inhaled (Meehan, 2002). Only agricultural lime should be used to increase alkalinity in a pond once it is stocked with fish as the hydrated and quick limes are not safe for use with live fish.

Lime should be applied to a new pond at the following rates:

a) Hydrated lime 50 kg per acre.
b) Quick lime 85 kg per acre.
c) Agricultural lime 100 kg per acre

About a week after lime application, farmers should spread 2 tons per acre of fresh cow or buffalo manure over the pond bottom. If poultry droppings are used, the amount should be reduced to 250 kg per acre. The pond should then be filled with water. Wild fish such as 'sowl' or 'muli' (*Wallago attu*) are predators and the farmer should make every effort to keep wild fish out of his pond by drying the pond, with application of quick lime, screening the incoming water. It is also desirable that all weeds should be removed from the pond, as weeds do not serve much purpose in a pond and can create many problems.

3.1.2 Stocking

Stocking a pond means releasing in the pond an adequate number of the selected fish species, which are of proper size. The stocked fish should grow within a given time according to reach the "market size". In fish farming, the stocking rate is based on the size of the fish required for the market and the total weight of the fish that a pond can be expected to grow (carrying capacity).

In Pakistan at present, farmers will receive the best price for carp that have reached a size of between 1 and 1.5 kg; fish smaller than 750 grams receive a somewhat lower price. Fish of 2 kg or more receive the same price as a fish of 1 to 1.5 kg or a slight premium. Therefore, a farmer should strive to sell his fish at the smallest size acceptable to the consumer, as it reduces risk and allows the potential for another crop to be produced in the ponds.

Note: The quality and condition of the fish stocked should be satisfactory. If wild fish get access to the ponds, the yield might be reduced considerably. Such species should be controlled by screening the in-flowing water properly.

Fish density in the pond is important. Understocking will result in incomplete utilization of the food available. Overstocking will result in slow growth and stunting of fish at smaller sizes that may be unmarketable. Stocking rate depends upon the productivity of the pond, the species of fish to be reared, the use of prepared feeds and the use of extra aeration.

80:20 Polyculture Concepts

The concept of 80:20 pond fish culture is to raise crops of fish in the pond where approximately 80% of the harvest weight is composed of only one feed-taking high value species with high consumer demand and approximately 20% is composed of “service species” such as filter feeders that help clean the water and/or predaceous fishes that control unwanted reproduction, wild fish and other competitors. Many farmers believe the 80:20 system provides higher yields and higher profit than monoculture or with other relative densities of fish.

3.2 Cage Culture in Inland Waters of Pakistan

Cage fish culture is a method of raising fish enclosed on all sides and the bottom by materials that hold the fish inside while permitting water exchange and waste removal into the surrounding water (Beveridge, 1987). The practice of cage culture is increasing all over the world. The site location and water quality is critical for success with cage culture.

Cages should be sited within topography that allows free water flow with a current in the range of 0.5-1.5m/sec. Fish cages should not be sited in the path of the inflow of any pollutant. Preferable water depth is 3-5m, but better not to exceed 12m as greater depths are more likely to develop anaerobic hypolimnetic conditions and lake turnovers that can suffocate fish. Areas where cages will be sited will benefit from light breezes and winds as small waves increases aeration and mixing. However, heavy winds may damage structures are break the cage loose from moorings. Thus, the bays or areas with some protection may be preferred.

Cage Design and Construction

Cages in Pakistan are typically rectangular or square shape, but cylindrical cages are also used sometime. Traditionally, fish cage size ranges from $100m^3$ to $1,000m^3$. Cages can also be made from readily available construction materials such as PVC pipe, wood, bamboo, or steel. Cages can be used in both freshwater and marine environments. A fish cage is simply a screened enclosure of plastic-coated wire, plastic extruded mesh, nylon, or polyethylene netting.

Picture: Floating cages in small mini dam, named Salli Dam, used for feeding trial in 2012. Photo courtesy R.S.N. Janjua, SoyPak (ASA/WISHH)

Fish Species and Stocking for Cage Culture

Fishes suitable for cage culture must tolerate crowding, accept artificial feed, exhibit good growth rate, be readily available for stocking, and have a good market prices. The natural and nutritional habitat of the fish species is very important. Fresh water fishes, such as *Clarais lazera*, *Clarias gareipinus*, *Orechromis niloticus*, *Cyprinus carpio* are successfully cultured in cages. A typical recommended stocking density for these catfish, tilapia, and common carp is 80 fish/m^3. Another recommended stocking density for beginning farmers is the number of fish that will collectively weigh 150 kg/m^3 when the fish reach a predetermined harvest size (Schmittou, 1991). The smallest recommended fingerling size for stocking is 15 g. A 15-g fish will be retained by a 13-mm bar mesh net. Larger fish can also be stocked into cages. Survival rates in well-placed and well-managed cages are typically 98 to 100 percent.

Unless greater mortality is expected, no adjustment is needed to calculate stocking density.

An example of how to calculate the number of fish to stock per cage follows:

Assume that a farmer wants to harvest fish weighing 500 g from a 1-m^3 cage.

$$\text{Number to stock} = \frac{\text{total fish weight at harvest}}{\text{desired average fish weight at harvest}} = \frac{150\ \text{kg/m}^3}{0.5\ \text{kg}} = 300\ \text{fish/m}^3$$

In Pakistan, a cage volume of 100 m3 is typical. Technical possibilities and maintenance expertise will determine the depth of the nets. If the net is deep, special diving equipment will be required for daily or weekly checking of the nets and eventual repairs. If the depth does not exceed 3 to 3.5 m., repairs and maintenance can be made through free diving.

3.3 Raceway

A raceway, also known as a flow-through system, is an artificial channel used in aquaculture to culture aquatic organisms. Raceway systems are among the earliest methods used for inland aquaculture.

Picture. Intensive tilapia culture in raceways in Arizona, USA.
Photo courtesy: R.S.N. Janjua. SoyPak (ASA/WISHH)

A raceway system usually consists of rectangular basins or canals constructed of concrete and equipped with an inlet and outlet. A continuous water flow-through is maintained to provide the required level of water quality, which allows animals to be cultured at higher densities within the raceway. Feeding and harvesting is easier in raceway systems. Disease treatment is more manageable in the raceways. Freshwater species such as trout, catfish, and tilapia are commonly cultured in raceways.

Picture: PK Team-1 visiting intensive circular tanks tilapia culture at University of Arizona, USA. Photo courtesy: R.S.N.Janjua, SoyPak

3.3.1 Site Selection, Shape and Size

Good quality sites for raceway culture are where water is available in abundance. A gradual slope of the topography is often useful. Raceways constructed near freshwater springs are ideal. According to availability of land and species cultured, various shapes and sizes of raceways, generally circular and rectangular, are common. Rectangular raceways, also called linear raceways, allow water to enter from one end and exit from other end. These were the first type to be developed and are used all over the world. In a typical commercial system, a single raceway can be up to 30 meters long and two meters wide. A raceway farm for freshwater finfish usually has a dozen or more parallel raceway strips built alongside each other, with each strip consisting of 15 to 20 or more serial sections (Ali, 1990). The Swat Valley in Pakistan has several small trout farms with single or paired raceway configurations.

Water Flow

The water flow rate in a raceway system needs to be sufficiently high to meet the respiratory (dissolved oxygen) requirements for the species concerned and to flush out metabolic wastes, especially ammonia. However, in most cases it is necessary to frequently clean raceways. The simplest way is to lower the water level in the raceway units, which increases the speed of the water current, and then herd the fish together until the waste is flushed from the raceway. Generally, the water should be replaced each hour. However, the optimum flow-through rate depends on the species, because there are differences in the rates at which oxygen is consumed and metabolic wastes are produced. For example, trout and juvenile salmon are less tolerant of degraded water quality and require a more rapid water turnover than catfish or tilapia.

TYPES OF FISH CULTURE SYSTEMS

There is a continuum of fish culture systems:

a) Extensive
b) Semi-intensive
c) Intensive

4.1 Extensive

In extensive fish farming, the inputs of management are minimal. The most extensive systems are little more than managed fisheries with little additional input. As the systems become more intensive, the inputs increase. Unmanaged spawning will be replaced with stocking. Fertilizer will be added to increase productivity. Harvest rate may be increased. Water exchange may be increased.

In Pakistan, in a typical extensive system, the fish feed entirely from the food web within the pond, which may be enhanced by the addition of fertilizer or manure (organic and inorganic). Ponds (natural or artificial) and lagoons are fertilized to promote the presence of phytoplankton (microscopic plants), zooplankton (mostly small crustaceans), and aquatic vegetation that form the base of the aquatic food pyramid (Ali, 1995). This encourages the development of marketable animals at a higher yield than that of the natural ecosystem. Stocking density is usually under 2,000 fish per acre and yield is less than 1,000 kg per acre. Extensive farming of carp and tilapia is common in Asia and Africa. Early carp farms in Pakistan utilized extensive systems which have evolved to be more semi-intensive.

4.2 Semi-Intensive

In semi-intensive culture systems, natural food and commercial/supplementary feeds are used, stocking rate and water exchange may be increased. This means the fish can grow faster and/or to a larger size or at a greater stocking density. Grass carp is the farmed fish species with the highest global production tonnage and is farmed across Asia in semi-intensive pond systems. Rohu is used most commonly in Pakistan. In semi-intensive farming, the carps are stocked in ponds, often with other carp species (major carps and/or Chinese carps), where phytoplankton, zooplankton, aquatic weeds and terrestrial grasses form the main feed and commercial feeds, usually in the form of mashes consisting of grain by-products, may be broadcast on the water surface for additional nutrition and to further fertilize the system. The total stocking density is 750-3,000 fish per hectare (i.e. 13-3 square meters per fish).

4.3 Intensive

Intensive pond culture of fish is mainly carried out in carefully designed ponds with a water depth of 1- 2.5 meters. Intensive fish farming utilizes a high degree of management and inputs that can include higher stocking densities, an external food supply of carefully formulated feeds containing complete nutrition, and careful water quality monitoring and management.

Adjustments may be made to water quality by adding extra aeration, altering pH with minerals, changing feed formulation or even bacterial or enzyme addition. Super-intensive systems utilize plastic pond liners, constant aeration, computer controlled monitoring of multiple parameters and very high stocking and harvest rates. Intensive systems, as well as nursery systems often utilize concrete tanks or raceways (Ali, 1990). These are beneficial for small fish production (fry and fingerlings) or for very intensive production as they can be carefully managed and harvested, especially if turnover of fish will be frequent. Fiberglass tanks and raceways can be even more useful in these situations as they are very easy to keep clean, are light and movable, and can be easily repaired if broken or if plumbing changes are needed. However, both concrete and fiberglass are expensive compared to digging a pond. An alternative that is more frequently used today are temporary tanks built from heavy duty plastic liners supported with bricks or cement blocks for shallow raceways and with posts and metal or wood frames for deeper raceways.

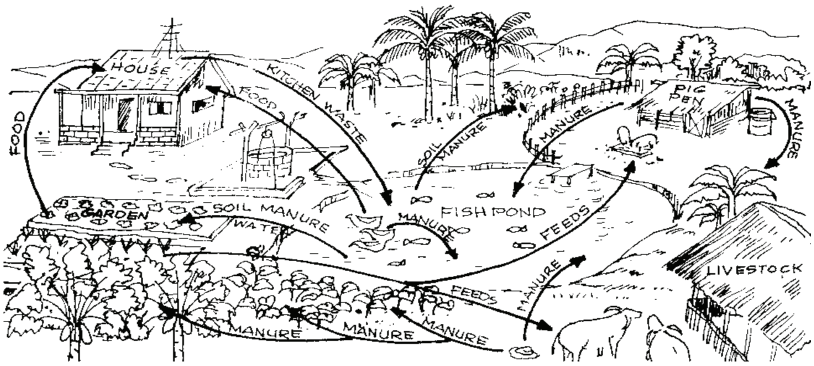
5. INTEGRATED AQUACULTURE

About 2000 years ago, the first recorded integrated aquaculture system (rice-fish farming) was developed in China. The term "Integrated Aquaculture" is used to describe the integration of monocultures through water or nutrient transfer or refers to the concurrent or sequential linkage between two or more farming activities, of which at least one is aquaculture. A variation on this is integrated multi-tropic aquaculture in which the by-product (wastes) from one species is recycled to become input (fertilizer, food) for another. It also enables effective utilization of available farming space for maximizing production. The rising costs of protein-rich fish food and chemical fertilizers, as well as the general concern for effluent releases and energy conservation, have created interest in these systems. Integrated fish farming can be broadly classified into several categories:

i) Agriculture-fish system which includes rice-fish integration, horticulture-fish system, mushroom-fish system, and aquaponics systems.

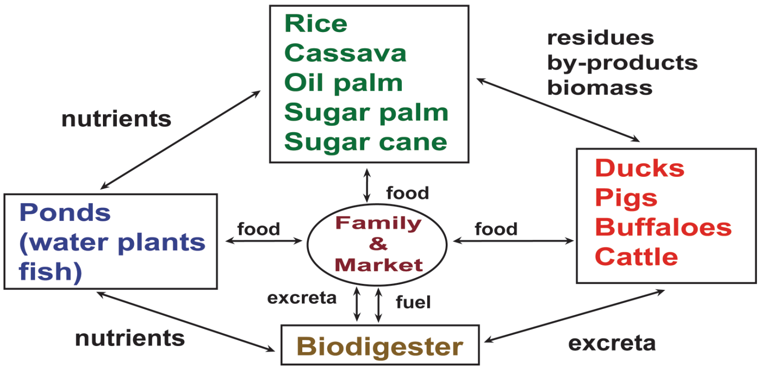
ii) Livestock-fish system which includes cattle-fish system, pig-fish system, poultry-fish system, duck-fish system, goat-fish system, rabbit-fish system.

iii) Marine multi-trophic systems which include consumers of feed (fish, crustaceans, etc.) filter feeders and detritivores (bivalves and worms) and primary producers (phytoplankton, seaweeds, halophytes).



Fish culture in combination with plant crops or livestock can be a complex venture and should provide a higher farm income. It increases productivity on small land-holdings and increases the supply of feeds for the farm livestock. The scope of integrated farming is considerably wide. Ducks and geese are raised in a pond, and pond-dykes are used for horticultural and agricultural crop products and animal rearing. These systems provide a variety of meat, milk, eggs, fruits, vegetables, mushroom, fodder, and grains, in addition to fish. Hence, this system provides better production; provides more employment; and improves the socio-economic status of farmers for betterment of the rural economy.

The integrated farming system



4. SOIL & WATER QUALITY

5.1 Soil Quality

There are two primary considerations regarding soil quality

i) Physical quality

ii) Chemical quality

i) Physical Quality

The nature of a particular soil depends on its physical properties and nutrient content. Soil has particles of various sizes, namely sand, silt, and clay. Among these three types, sand is the largest and clay is the finest. The relative amount (percentages) of these three sizes is known as soil texture. Pond soil should have enough clay to hold water and prevent erosion of pond dikes. 100% clay is not suitable for pond construction since the pond dikes crack when exposed to drying conditions. Once the dikes are cracked, they get weak and water may leak out (Ali, 1995).

How do you determine the ability of soil to hold water in your pond?

A percolation test is a common method to determine how soil will hold water. Dig a hole/pit as deep as your waist. Early in the morning, fill it with water to the top of the hole/pit. In the evening, you will notice some of the water has been absorbed into the soil and the water level has gone down. Fill the hole/pit with water again to the top and cover the hole/pit opening with some leafy branches or some boards. Next day, examine the water level in the morning. If most of the water is still in the hole/pit, the soil is good to hold water in your pond.

The nutrient content of soils is determined by a soil fertility test. A soil fertility test involves principally, the determination of the various nutrients present and the levels of availability of each of the nutrients.

Nutrient problems are diagnosed from the tests. Improvement of the nutrient levels of pond soils involves the addition of fixed amounts of lime, fertilizer, and manures.

ii) Chemical Quality

Soil quality is an important factor in fish pond productivity as it controls pond bottom stability, pH, and salinity. This means that pond soil can regulate the quality of the overlying water. Fish pond soil should not be acidic because it reduces productivity. For example, growth of algae, which is a natural food for many fishes and good for a pond environment, may be greatly reduced. Acidic soil makes acidic water. It retards growth of fish and causes a stressful situation, favoring infections and diseases (Ali, 1995).

Acid sulphate soils, pH equal to or less than 5, are not good for pond construction. Pale yellow mottles are indicative of acid sulphate soil. Waterlogged, unconsolidated soil, tends to become more acidic upon drainage and exposure to air.

How do you identify potential acid sulphate soils?

i) Take a dry soil ball and break it. Examine the inside areas of the broken soil ball. If you see yellow color mottles and/or streaks, the soil may contain potential acid sulphate.

ii) Take a handful of the soil to be tested. If the sample is dry, moisten it. Make the soil sample into a cake of one cm thick. Put the moist cake into a thin polythene bag and seal it. After one month, measure the pH of the soil in the cake. If the pH has dropped below 4, the soil is a potential acid sulphate soil. (If you need to identify potential acid sulphate soils quickly, soil samples will have to be taken to a laboratory)

5.2 Water Quality

Fish is an important cash crop in many regions of world and water is the physical support in which they carry out their life functions. Water quality is determined by various physico-chemical and biological factors which directly or indirectly affect its suitability for the distribution and production of fish and other aquatic animals.

There are related categories of water quality

i) Physical Characteristics
ii) Chemical Characteristics

5.2.1 Physical Characteristics

Source

In Pakistan, there are a variety of water sources used for aquaculture. In the Swat Valley, most of the trout farms use water diverted from streams through the farm and then returned to the stream. In Punjab, most of the farms use well water (borehole) that is pumped from relatively shallow aquifers (usually less than 10 meters). In Sind, farms use a mix of well water, watershed runoff and streams, and some from managed irrigation systems. With well water there is rarely any need to screen the water for any particulates, parasites or predators (Wetzel, 1983). For streams or canal supplies, screening or even a settling basin and then screening may be advisable. For fast moving waters, silt, sand, gravel and branches may be introduced, so a settling or "head tank" may be a worthwhile investments. This should trap and allow settling for heavy materials and floatation of lighter materials. Screening with water passing through a gate or weir structure before entering a raceway, tank or pond will protect the fish. Screens can be constructed of wire mesh with a metal or wooden frame. The frame and screen should be removable for cleaning. For removal of biological contaminants, a sock or tube filter is advisable. These socks are available in lengths of up to 10 meters and can effectively filter out particles and biologicals to 100 microns. The filter sock should be removed from the inlet pipe on regular basis for cleaning and disinfection. An additional sock that will allow for rotation and sun drying is preferable.

Temperature

Temperature influences almost all the physiological activities of aquatic animals and plants such as respiration, growth, feeding, breeding, moulting, gamete formation, and disease resistance. In general, the suitable temperature range for tropical fish is 25 to 30^0 C.

Suspended Solids

Suspended solids include a variety of materials including clay, silt, algae, organic matter, fines from feed, bacteria, and fish excrements. Fish wastes can be a serious problem for a fish culture system. These wastes often irritate the fish's gills but can cause several problems as effluents may overwhelm the capacity of a pond's ecosystem to reduce the load. Nitrifying and heterotrophic bacteria require dissolved oxygen and excessive suspend solids may deplete these supplies (Wetzel, 1983).

Plankton

Plankton is often beneficial to fish. Phytoplankton (cyanobacteria and several kinds of algae) not only produces oxygen, but also provides a food source for zooplankton and filter feeding fish/shellfish. Phytoplankton also uses ammonia produced by aquatic animals as a nutrient source. Zooplankton is a very important food source for fry and fingerlings such as hybrid striped bass and yellow perch and all life stages of some carps and tilapias. However, excessive amounts of algae can lead to increased total of respiration during the night thereby consuming all available oxygen. Excessive phytoplankton buildups or "blooms" which subsequently die will consume even more oxygen. Wide swings between day and night dissolved oxygen levels can lead to dangerously low oxygen concentrations during the predawn hours when pond dissolved oxygen levels tend to be at their ebb.

Clay

Most clay creates a problem of turbidity in a fish pond. Turbidity restricts light penetration and limits photosynthesis. Sedimentation of soil particles may also smother fish eggs and destroy beneficial communities of bottom organisms such as bacteria. Turbidity is a measure of the degree to which the water loses its transparency due to the presence of suspended particulates. The more total suspended solids in the water, the murkier it seems and the higher the turbidity. Turbidity is measured in NTU: Nephelometric Turbidity Units. The instrument used for measuring it is called nephelometer or turbidimeter, which measures the intensity of light scattered at 90 degrees as a beam of light passes through a water sample. In aquaculture ponds we often estimate the turbidity with a secchi disk. This is a black and white disk that is dropped in the water attached to a rope or pole with markings for depth. The depth that the disk reaches before it disappears from sight is the secchi depth and should be noted.

5.2.2 Chemical Characteristics

Photosynthesis

Photosynthesis is one of the most important biological activities in pond aquaculture. Many water quality parameters such as dissolved oxygen, carbon dioxide, pH cycles, and nitrogenous waste products are regulated by the photosynthetic reaction in phytoplankton. Simply stated, photosynthesis is the process by which phytoplankton uses sunlight to convert carbon dioxide into a food source and to release oxygen as a by-product.

Chlorophyll of Plants + Carbon Dioxide $\longrightarrow$ Sunlight = food + oxygen

In addition to supplying oxygen in fish ponds, photosynthesis also removes several forms of nitrogenous wastes including ammonia and nitrates.

Dissolved Gases

The dissolved gases of concern for aquaculture are oxygen, carbon dioxide, nitrogen, and ammonia. Concentrations are measured in parts per million (ppm) or milligrams per liter (mg/1); both units of measure are the same. (One ppm or mg/1 is essentially the same as one pound added to 999,999 pounds to total 1,000,000 pounds) (Wetzel,1983).

Oxygen

The dissolved oxygen in water is one of the most important water quality parameters to maintain a healthy pond environment. The amount of oxygen that can be dissolved in water decreases at higher temperatures, salinity, and altitudes. The dissolved oxygen level of 5.0 to 6.0 mg/liter (essentially equal to 5.0 to 6.0 part per million, ppm) is adequate for most fish to be reared in ponds in Pakistan. It should be remembered that the concentration of oxygen in air is 20% or 200,000 mg/liter and that diffusion of oxygen from air to water is the most important source of new oxygen into a pond.

Carbon Dioxide

Carbon dioxide (CO_2) is commonly found in water from biological respiration or water sources originating from limestone bearing rock. Water supporting good fish populations normally contains less than 5 ppm of free carbon dioxide. In water used for intensive pond fish culture, carbon dioxide levels may fluctuate from 0 ppm in the afternoon to 5-15 ppm at daybreak. While in recirculating systems, carbon dioxide levels may regularly exceed 20 ppm. Excessively high levels of carbon dioxide (greater than 20 ppm) may interfere with the oxygen utilization by the fish. Tilapia has a tolerance range of CO_2 is 0-30mg/l.

There are two common ways to remove free carbon dioxide. First, with well or spring water from limestone bearing rocks, aeration can "blow" off the excess gas. The second option is to add some type of carbonate buffering material such as calcium carbonate ($CaCO_3$) or sodium bicarbonate (Na_2CO_3).

Nitrogen

Dissolved gases, especially nitrogen, are often measured in terms of "percent saturation." A gas super-saturation level above 110% is usually considered problematic. Gas bubble disease is a symptom of gas super-saturation. Nitrogen is the most common supersaturated gas, but oxygen and carbon dioxide can also occur in ponds. The signs of gas bubble disease can vary. Bubbles may reach the heart or brain, and fish die without any visible external signs. Other symptoms may be bubbles just under the surface of the skin, in the eyes, or between the fin rays. Treatment of gas bubble disease involves sufficient aeration to decrease the gas concentration to saturation or below.

Ammonia

Ammonia is a dissolved gas present naturally in surface and wastewaters, and in some well waters. It is the major nitrogenous waste product of fish and also results from the decomposition of organic matter. It is quite soluble in water, especially at low pH, and ordinarily is removed by plants or bacteria. Ammonia in water will be present in ionized and un-ionized forms, with the ratio dependent on pH and temperature (Pillay, 1990). As the pH increases, there is an increase in un-ionized ammonia, which is toxic to the fish. Tilapia tolerance range is up to 0.4mg/l before chronic inflammation appears. Other fishes tend to be more susceptible and likely to be impacted at lower levels.

pH

pH value is a measure of hydrogen ion content and will tell you whether water is acidic or basic. The pH of pond water is a good indication of health of fish and pond environment. For most fish a pH value between 6 and 8.5 is best.

Alkalinity

Alkalinity and hardness are both important components of water quality. The most important components of alkalinity are carbonates and bicarbonates. Most aquatic organisms can live in a broad range of alkalinity concentrations. The desired total alkalinity level for most aquaculture species lies between 50-150 mg/L $CaCO_3$, but no less than 20 mg/L.

Hardness

Total hardness is a measure of the calcium and magnesium concentrations in water. Other divalent ions such as aluminum, iron, manganese, strontium, and zinc ions (those with 2+ charges) also contribute to total hardness but are usually present in insignificant amounts. The amount of calcium hardness is an important consideration in pond fertilization because higher rates of phosphorus fertilizer are required at higher calcium hardness concentrations. Hardness values of at least 20 ppm should be maintained for optimum growth of aquatic organisms (Pillay, 1990).

Quantity

The beginning aquaculturist usually underestimates the quantity of water required for commercial production. It is generally accepted that a minimum rate of 13 gallons per minute (gpm) is required for each surface acre of ponds. With this in mind, a 100- acre fish farm will need to have wells capable of producing 1,300 gpm of water.

Such large volumes are required to replace water lost to evaporation and seepage, which tend to be high in Pakistan farms. In addition, the farmer may have several ponds to fill quickly during the spawning season.

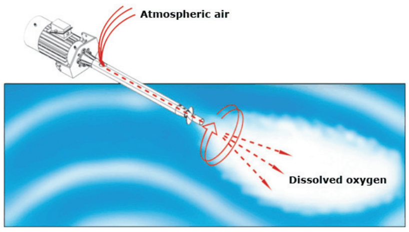
Aeration

Adding dissolved oxygen to an aquaculture system is one of the most important management tools a farmer has. In fact, aeration achieves many beneficial goals at the same time that oxygen is added to water. For example, depending on which physical action is undertaken for aeration, the side benefits include releasing carbon monoxide and carbon dioxide and ammonia, stirring and mixing water, which brings low oxygen content to the surface and high oxygen content surface waters to lower levels. Aeration, and the mixing that comes with it, will also disrupt thermoclines (temperature stratification) that will improve water quality, reduce anaerobic conditions in sediments and generally make more of the pond volume available to the fish.

There are several categories of aeration systems. The most basic is just a water drop or water fall from one level to another. This can be enhanced by breaking the smooth laminar flow of water with something that will cause a splash, rocks, boards, etc. A simple device called a low head aerator is basically a pipe with perforated plates inside that maximizes the opportunity for unwanted gases to diffuse out of the water and oxygen to diffuse in. The more active aerators include an impeller type aerator that throws water up into the air with a fountain type effect. A paddlewheel aerator rotates with arms that splash the surface, push the water ahead and throws some off the paddles as they rotate out of the water. An aero2 is a hybrid aerator that also works as an aspirator, sucking air down into a tube, and a mixer with a propeller shaft in the center. The rapidly spinning shaft with the propeller on the end forms a dense cloud of small air bubbles to help with pond water mixing. Yet another category of aeration systems are diffusers. These can be plastic, eramic, porous stone or other manufactured materials that will release fine bubbles.

A. Fountain aerator

B. Paddlewheel



C. Aspirator

These can be just a centimeter or two in length or as long as 100 meters. In each case air or oxygen under pressure is forced though the material so that is forms bubbles with rise to the surface. With plenty of surface area they diffuse oxygen into the surrounding water and entrain water as the bubbles rise, bringing water up to the surface.

One of the most important factors to remember is that the greatest amount of oxygen exchange in ponds actually occurs at the surface. The most important function of all aerator systems is to bring oxygen poor water from the below the surface to the surface where it can contact atmospheric air. Remember that air has 20% oxygen or 200,000 ppm. Pond water will only have 6 or 7 ppm at saturation.

So, the more water that comes to the surface and has the chance to absorb oxygen from the high concentration in the air to the relatively low concentration in the water, the more effective will be the aeration system.

5. QUALITY OF FISH SEED

Genetic management and improvement of aquatic stocks for aquaculture and biodiversity is still at a very early stage in Pakistan. Locally run selective breeding programs with pedigreed stocks are still being developed. A novel aspect of aquaculture genetics is the ability to produce mono-sex populations (all-male or all-female), where one sex performs better than the other or to limit reproduction among culture fish where this reduces performance. Techniques for mono-sex production have been developed and applied in projects in several countries for species of tilapias and cyprinids.

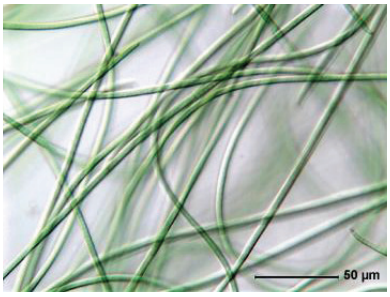
6. ALGAE MANAGEMENT – WATER QUALITY, TASTE AND ODOR ASPECTS

More than 30,000 species of algae have been described, and scientists have found that algae have been around for at least two billion years. They occur in virtually every habitat on earth, where water and sunlight are found, even if the water is only present for a very short time. They can survive severe environments, from icy mountain glaciers to boiling hot springs to excessively salty water. However, for the purposes of this handbook, we will limit ourselves to algae that grow in ponds, lakes, and streams.

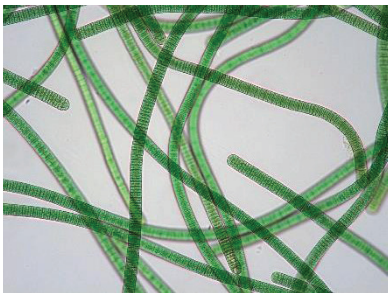
Algae can float freely in water, coloring it green, brown, yellow, or even red, or they can coat the sides of a pond with a green or brownish slime. In the ocean, they provide the food base for most marine food chains. Without algae, our waters would not sustain life and mankind would not benefit from its countless qualities and boundless beauty. However, in very high densities, called algal blooms, algae not only discolors water but can also out compete or poison other life forms in it.

The most common kind of algae is called planktonic algae. These are single-celled or small colonies that create most algal blooms and impact pond owners worldwide. They reproduce rapidly and can be green, brown, or red in color. They can be toxic to animals and can give water and fish unpleasant tastes or odors.

The most common cause of off-flavor or taste and odor in lakes, ponds, rivers, and reservoirs is from cyanobacteria, also called blue-green algae. The two most common are filamentous cyanobacteria, *Oscillitoria* and *Lyngbya,* which produce geosmin and methyl-isoborneol.



Oscillitoria sp.



Lyngbya sp.

These are two small volatile organic compounds that are noxious but not known to be harmful. As the compounds are produced by the cyanobacteria (blue-green algae) some is leached into the water and some remains in the cells in the filament. Fish can absorb the compounds from the water or from consuming the filaments. The compounds are transferred in the blood and will be found in the flesh of the fish. The farmer may detect the smell in the water, or more likely by tasting the fish.

Farmers and processors have a simple test for off-flavor in fish. The suspect fish is filleted and the fillet is put into a small paper bag, rolled up and placed into a microwave oven for 30 seconds. The bag is removed and unrolled. An off-flavor fish will either have a grassy or muddy smell or taste. The quality of the fish can be recovered in 24 to 48 hours by placing the fish in clean water and withholding feed. The geosmin and methyl-isoborneol are small volatile organics that quickly degrade and dissipate. The fish will be ready to process and/or consume in just a couple of days after removal from water that contains the blue-greens (cyanobacteria).

AQUATIC PLANTS

Aquatic plants are a natural part of most pond and lake communities and provide many benefits to fish, wildlife, and people. The natural balance of the aquatic ecosystem is disrupted when invasive and nuisance levels of aquatic plants dominate these waters. The negative impacts that can result include: declining fish and wildlife habitat, poor water quality, diminished recreation (fishing, swimming, boating), and reduced property value.

Most aquaculture ponds lack aquatic plants as the pond bottoms tend to be limed, the fish tend to eat or pull up the plants, and the phytoplankton tend to absorb the light and nutrients. Also, aquatic plants can be a nuisance when trying to harvest the pond and process the fish. Aquatic plants fall into three functional groups with similar characteristics.

1. Submerged aquatic vegetation - These plants have stems and true leaves. Almost all the plant is submerged. Sometimes submerged plants have a few floating leaves and reproductive structures at the water surface.

2. Floating plants - Plants that have most of their leaves and flowers floating on the water surface. They range from *Azolla* and duckweed (tiny floating plants) to water lilies.

3. Emergent plants - Sometimes called wetland plants, they are rooted in sediment or moist soil and have most of their stems and leaves extending above the water surface. Cattails are a good example of this category of plants.

Integrated Pest Management (IPM)

Fish farms are subject to a number of pests. These can include parasites, snails, crabs and other crustaceans, aquatic plants, algae, wading birds, diving birds, fish eating snakes and lizards, and various mammals. In all cases there are a number of options for controlling the pests, mechanical or physical, chemical means, and biological means. Integrated pest management plans recognize that any one of these methods is unlikely to succeed by itself or would be prohibitively expensive. IPM plans are put together in advance with the understanding that pest are virtually a given in any kind of agriculture and that advance planning will reduce costs and losses. An IPM plan takes into account the various methods available and utilizes the benefits of the various techniques to come up with the most cost effective control strategy. This may also include a certain fraction of loss if the cost is low enough.

For example: enclosing an entire pond with a structure to support bird netting would be effective, but cost prohibitive. An IPM plan for birds, might include a single monofilament line barrier 20 cm high and 50 cm on shore from the water to keep out wading birds, A few dozen monofilament lines with streamers crisscrossing the pond will dissuade many of the diving birds. A dog or two on the farm will also chase away certain birds. Staff can also chase away persistent pest birds.

References

Ali, 1995. *Basic Concepts of Ecology*. Naseem Book Depot, Hyderabad. PP. 311.

Dill, W. A. and Pillay, T. V. R., 1968. Scientific Basis for the Conversation of non-Oceanic Living Aquatic Resources. *FAO. Fish. Tech. Paper*, 82.

Huet, M., 1994. *Text book of fish culture* (Breeding and cultivation of fish (Second Edition) Fishing News Books Publishing House. PP. 434.

Martyshev, F.G., 1973. *Pond Fisheries*. Prem Printing Press, Lukhnow. PP. 474.

Meehan, W. E., 2002. *Fish culture in ponds and other inland waters* .H. R. Publishing House pp. 281.

Narejo, N. T., 2006. Study on the food and feeding habit of Palri, *Gudusia chapra* (Hamilton) from fishpond in District Thatta, Sindh, Pakistan. *Pakistan J. Zool*., 38 (2): 93-98.

Pillay, T.V. R., 1990. *Aquaculture* (Principles and Practices). Blackwell Publishing House PP. 575.

Wetzel, R. G., 1983. *Limnology* (Second Edition) Saunders College Publishing House PP 767.

CHAPTER - III

BIOLOGY OF FRESHWATER FISHES FARMED IN PAKISTAN

Author
Dr. Muhammad Afzal
Associate Professor / Chairman
Department of Zoology and Fisheries
Faculty of Sciences, University of Agriculture
Faisalabad - Pakistan
E-mail: afzal160@yahoo.com

Co-Author
Dr. Abdul Mateen
Lecturer
Department of Zoology and Fisheries
Faculty of Sciences, University of Agriculture
Faisalabad – Pakistan
E-mail: mateen117@uaf.edu.pk

INTRODUCTION

More than twenty-five thousand finfish species have been reported from different ecosystems of the world. Freshwater fishes constitute approximately 8,000 species, inhabiting lakes and rivers. Of these 8,000, only four hundred species are considered to be commercially important which include cultured and wild species (Nelson, 2006). Approximately 193 native fish species are reported in freshwaters of Pakistan, including some important groups such as loaches, carps, catfishes (Mirza, 2009; Zafar *et al*. 2013). Among them twenty-eight fish species are native to the cold waters of Pakistan. Most of the snow carps are restricted to Trans Himalayan region of the Indus System. Exotic brown trout is common in rivers and streams especially in Northern areas, Khaiber Pakhtoon Khah (KPK) and Azad Jammu and Kashmir (AJK). Mahseers and Schizothoracines (mountain carps) are becoming rare due to over-fishing and the disappearance of their spawning grounds. About thirty species belonging to the following different groups are commercially important;

- Carps
- Snakeheads
- Tilapias
- Catfishes

Carps are the biggest group of freshwater fishes in Pakistan and can be further divided into two major groups.

(A) Indigenous major carps
(B) Exotic carps

- **Indigenous Major Carps**
  Pakistani major carps include *Labeo rohita* (Rohu)*, Catla catla* (Thaila) and *Cirrhinus mrigala* (Mrigal). These species are also commonly cultured in many South East Asian countries.

Labeo rohita

It is the most important species of major carps, commonly known as Rohu or Dumbra but sometimes locally called as Rohi.

Labeo rohita (Rohu)

The body of this fish is elongated and symmetric and covered by cycloid scales, except the head. The snout is depressed and mouth is terminal with small thickened fringed upper lip. A small pair of barbels is present in this fish. Rohu is a eurythermal species but does not survive temperatures below 14°C and shows best growth between 27°C to 30°C with oxygen-rich water. Rohu spawn in shallow and marginal areas of river in natural waters. Rohu start feeding on the third day of its life on natural food composed of zooplankton and phytoplankton and, after 10th day of its life, it can be weaned on artificial feed. Rohu is a column feeder and primarily subsists on algae and biofilms growing on leaves of submerged vegetation, with occasional intake of decayed organic matter.

It can mature in two years in a well- cared environment but takes about 4-5 years in the wild. At maturity, this fish is of average 30 cm in size. Naturally, rohu spawns during the monsoon that normally lasts from April to September. At maturity, dorsal surfaces of pectoral fins of males become rough and ooze out few drops of milt when the body is slightly pressed on the ventral side. Females show an inverted pinkish or red vent when they are fully gravid. The fecundity depends upon fish size and ovary weight of fish. A 2-2.5 kg female fish can lay out approximately 25,000 eggs. Eggs are non-adhesive, demersal, whitish, and transparent. Eggs measure 1.5 mm in diameter but, after fertilization, they absorb water and swell up. Hatching occurs 28-32 hours after fertilization at 27-29°C.

Under normal culture conditions, rohu grows to 35-45 cm total length and 700-800 g total weight in one year. It is predominantly cultured in ponds all over the country in polyculture with other herbivorous fish species, where it contributes up to 35% of the total stocking and 23% of the total production.

Catla catla

Catla catla is commonly known as Catla, thaila or thail. It is the fastest growing major carp species.

Catla catla (Thaila)

Its body is laterally compressed with a comparatively bigger head. The body is covered with cycloid scales and snout is rounded without barbels. The caudal fin is forked.

Catla is a eurythermal species and shows best growth between 25-32^{o}C. It mainly consumes zooplankton and can consume phytoplankton in rare cases. Eggs are initially demersal but can become buoyant later on. In early stages of life, larvae are strongly phototactic and remain near the water surface. On the third day of life, larvae start feeding while their yolk sac persists. Fry consume zooplankton mostly, while adults feed only on surface and mid-waters. It also consumes insects, crustaceans, protozoa and rotifers. Algae and some other plant materials are also a considerable part of their food. Although it is primarily a surface feeder, it has the ability to feed at any level.

It matures as it approaches the age of two years. Females show maturity a little later than males. Spawning season starts with monsoon and lasts between May to August. *Catla* does not naturally breed in ponds, even when it is fully mature, because it requires natural fluvial conditions, so it is artificially induced to spawn. Eggs are silvery in color and are non-adhesive. They are spherical in shape with diameter of 2.0-2.2 mm. Hatching takes place 28-32 hours after fertilization.

Among all major carps, *Catla* has the lowest fecundity. It varies from 60,000 to 100,000 eggs per kg of body weight of fish. In ordinary conditions, *Catla* grows to 1-1.2 kg in its first year of age. It attains a maximum length of 1.83 meters with maximum weight of 60 kg. Growth is rapid during first three years and then slows down. Its production is sometimes 1.1-2.2 tons/ha in extensive farming systems.

Cirrhinus mrigala

This fish is commonly known as Mrigal, Mori or Morakhi. The body is symmetrical and streamlined with equal body depth and head length. Cycloid scales cover the body except for the head. Its mouth is broad with a single pair of small sized barbels.

Cirrhinus mrigala (Mori)

Fish at hatchling stage stay in sub-surface and surface level waters, while fry and fingerlings swim into deeper waters. Adults prefer to live in bottom waters. Depending on stocking density and management practices of a culture system, the species gains 600-700 g weight in the first year. Fry feed mostly on zooplankton and rarely on phytoplankton. Hatchling size ranges from 6-8 mm. Adults consume plant detritus and occasionally zooplankton.

Mrigal attains maturity earlier than Catla and rohu. Male fish mature sooner than females, mostly by the age of one year. Females are usually bigger in size than males. Like other carps, it rarely breeds in captivity, so it is induced artificially by hypophysation and/or other synthetic hormones. It can be induced to spawn from May to August but naturally it spawns in the monsoon season. Mrigal is a highly fecund fish. Fecundity increases with age, and ranges from 100,000 - 150,000 eggs/kg of body weight. Fish breed usually at 26 - 31ºC. Eggs are non-adhesive, transparent, yellow and demersal. In culture, mrigal grows to 0.6-0.7 kg in the first year, 1.5 kg in the second and 3.5 kg in the third year of its life, depending on stocking density and management practices. It is an esteemed table fish and has been grown all over Asia in mono- and polyculture systems. In extensive culture, its production is 1-2 tons/hectare, however, its growth rate is the lowest among major carps.

Exotic Carps

Ctenopharyngodon idella (Grass carp) *Hypophthalmichthys molitrix* (Silver carp), *Aristichthyhs nobilis* (Bighead carp) and *Cyprinus carpio* (Common carp) are of Chinese origin but have been cultured in Pakistan, India and almost all over the world for many years.

Ctenopharyngodon idella (Grass carp)

Its body is elongated with broad head and round snout. Body color is grey on the dorsal surfaces, while silvery on the ventral side. Body scales are of moderate size and are dark brown at the base. It is predominantly a freshwater fish but can survive in brakish water as well. It was imported to Pakistan to control aquatic vegetation in natural and man-made water bodies and to alleviate meat and protein deficiencies. Grass carp naturally lives in water bodies where submergent vegetation is present.

During the early life stages, grass carp mostly feeds on protozoa, rotifers, copepodes and benthic algae. As the fry grow up, they start eating phytoplankton and algae. Adult fish prefers higher vegetation and aquatic weeds as the major food source. Its excreta works as a natural fertilizer, indirectly serving as food for other fish species living in the same pond.

Grass carp reach maturity during the second to third years of age when it weighs 1.5 to 3 kg. Grass carp of 4 kg weight can lay 300,000 eggs. Grass carp, like the other major carps, does not breed in standing waters. It typically spawns from April to May and less often in later months of summer. Grass carp naturally breeds in rivers during monsoon. However, it can be bred artificialy like other carps. Grass carp is cultured world wide to control aquatic vegetation in natural and man-made water bodies. Due to its non-competitive nature, it is a suitabale candidate for polyculture systems. In the open market, it fetches the same price as Indian major carps of the same size.

Hypophthalmichthys molitrix

It is commonly known as silver carp. The body is silvery in color with dark fins. These fish have a keeled abdomen and body scales are of small size. Silver carp is a freshwater fish and native to China. Due to its fast growth, it has been introduced in many countries. In Pakistan, this fish was imported to enhance pond fish production per unit of area.

Hypophthalmichthys molitrix (Silver carp)

At early stages of its devlopment, this fish feeds on rotifers, copepods and zooplanktons. As fry grow up, they start feeding on phytoplankton and larger copepods. Diet of adult fish consists of phytoplankton. It is considered a fast growing carp that grows faster in rivers and flowing water bodies than in stagnent waters. This fish matures roughly at the age of one year and produces 200,000 eggs/kg of body weight. Eggs are smaller than other carps and are non-adhesive. Breeding season of silver carp starts in April and extends to September in Pakistan. It does not breed in stagnant waters but can be induced to spawn by hormonal manipulations.

Cyprinus carpio

Cyprinus carpio is of Europian origin, and in Pakistan is called common carp, gulfam or Europian carp. It is the most cultivated fish in the world in terms of meat production.

Cyprinus carpio (Gulfam)

The body of a common carp is elongated and its color varies with its environment. Its dorsal side is usually greenish-brown and its ventral side is usually yellowish golden. Common carp have two pair of barbels. Common carp are natives to the Asian Temperate Region especially China. For the purpose of aquaculture production, it has been introduced in many countries.

Common carp, at its early fry stage, feeds on diatoms, rotifers, *Euglena,* and crustaceans. As fry grow up, they start feeding on insects, and larger crustacea including *Daphnia* and *Moina*. Adult fish feed on decaying organic matter and bottom dwelling creatures like molluscs, tubificid worms, trichopterans and chironomids. Common carp mature much earlier than the other carps and attain maturity within first year of its age. Females lay approximately 100,000 eggs per kg of its body weight.

Common carp can breed twice a year, but normally breeds from Februray to March and then in October in the Northern Hemisphere. It can spawn successfully in both rivers and ponds without any hormonal manipulations. Eggs are adhesive and attach immediately to any substrate when released, especially floating grasses or similar artificial substrates. Common carp is very hardy and has excellent potential to survive and grow in a variety of environmental conditions under any production system. It accepts both natural and artificial feeds equally well.

Mahseer

Mahseers are considered the most important and beautiful game fishes of the South Asian subcontinent. Mahseers do well in mono- as well as in poly-culture. During the last four decades, extensive studies on the distribution, biology and fishery of the commercially important Mahseer have been conducted in different countries. Mahseers are mainly found in the hilly areas but sometimes descend onto the plains. Mahseers are the common name used for the fishes of genera *Tor*, *Neolissochilus* and *Naziri* in the family Cyprinidae, but more specific for genera *Tor* and *Naziri*. Mahseer are wide spread in many countries of South Asia including Pakistan, India, Afghanistan, Nepal, Bhutan, Myanmar, Bangladesh, Thailand, Indonesia and China. Genus *Tor* comprises of, at least, twenty-five species including *Tor macrolepis* whereas genus *Neolissochilus* has twenty-three species and *Naziri* two species.

Tor macrolepis (Mahseer)

In the Peshawar Gazetteer of 1896-97, fish weighing 100 pounds (45 kg) (probably mahseer) are mentioned in the Indus River at Attock (Mirza *et al*., 2002). Mahseers of two meter in length and 50 kg in weight have been recorded in Pakistan (Mirza, 1990). In 2002, Hakim Tahir Mehmood (local angler at Attock) caught a mahseer 4 feet and 8 inches in length and 32.3 kg in weight by rod and line from the Indus River at Brotha (Mirza *et al*., 2004). In addition to a sport fish, mahseers are also part of commercial food fishing and sometimes as an ornamental or aquarium fish.

Indus Mahseer *Tor macrolepis* is a widely distributed and commercially important species both of cold and warm waters. It inhabits hill streams, lakes and reservoirs like Tarbella Dam receiving snow melt water, but also warm water of the rivers Haro, Soan and Kabul etc. The mouth of *T. macrolepis* is suctorial and protrusible. It has two pairs of barbels. Typical bottom feeding habits are reflected by presence of mud and debris in its gut. Mollusks and insects larvae are also reported to be present in the gut.

Tor macrolepis is oviparous with the males commonly called "running males" during the breeding season. Males are virtually full of spermatic fluid during spawning and become mature earlier than the females. Even a male of only one hundred grams can become sexually mature in the second year of life. Females mature when they reach more than two hundred grams in size and are in the third year of life.

Sex identification is only possible when ripe females show a fully bulged and soft abdomen with a slight swollen pinkish vent. When the abdomen is slightly pressed, the mature females and males discharge eggs and milt respectively. No sharp differences in colors of the two sexes or roughness of the pectoral fins were noticed even in ripe males. The males are slimmer than the females.

The nesting habit, as exhibited by scooping of sand for deposition of ova, has been observed in *T. macrolepis* at its natural breeding grounds and also in captive conditions. During nesting, female *T. macrolepis* clears the bottom for depositing the freshly laid eggs. By this activity, rounded depressions are formed at the bottom surface. Such behavior is associated with the provision of maximum safety to the freshly laid spawn from natural predators.

The breeding mechanism of *T. macrolepis* involves a brief process of courtship. Two to four or even more males chase a fully ripened female. Mature females freely release eggs with a slight pressure on the abdomen. Ripe eggs are transparent with slight yellowish orange tinge. Stripping should be carried out in a shady place. The unfertilized spawn is kept away from any contact with water. Spawned eggs and milt should be kept cool, as it will prolong their life. Milt is obtained from the mature running males by gently pressing their bellies down to the vent. Males may be stripped several times. Milt is directly sprinkled on the eggs. Then the eggs and milt are gently mixed using a clean dry feather for one to two minutes. Eggs are then washed with water at least 2-3 times until they harden and get ready for shifting to incubators. Washing removes extraneous materials and excess milt. Water hardened eggs are shifted in incubators for hatching.

After two to three days post-fertilization, the embryo develops into a recognizable fish that lies inside the egg in a folded state. The squirming (twisting) motion can be seen due to the transparency of egg membrane. The baby manages to escape out through the weaker portion of the egg membrane. The fish is now called a hatchling or yolk sac fry. The hatchling cannot swim freely, as it has no well-defined fins. Its large yolk sac in the abdomen is also a hindrance for movement. For about three to five days, the hatchling leads a latent or quiescent life, remaining at the bottom of a hatching tray or tumbling in a hatching jar or bottle with occasional surface to bottom movement and vice versa. The hatchling is subject to infection of fungus and bacteria if water is not properly circulated and aerated.

By four days post hatching, a brisk swimming tendency is noticeable. Sac fry do not need to take food. A sac fry measures about 5-8 mm in total length. Pectoral and caudal fins are well formed after five days of fertilization; the yolk sac becomes fully absorbed, and develops into the swim-up-fry stage. After 7 to 10 days post hatching, swim-up-fry absorb all yolk content and start their free-swimming life. They exhibit a brisk movement with well-developed fins. Swim-up-fry come to the surface of the incubation tray to gulp air to encourage swim bladder inflation. Swim-up-fry start feeding on zooplankton. Up to this time the swim-up-fry are safe in the hatching tray. At this stage, swim-up-fry measures about 15-25 mm and will be moved to a hapa net in a pond or to a nursery tank. In traditional hatcheries, boiled egg yolk and white, and sometimes preferentially egg white only, are rendered into a fine paste by mixing with water; the yolk suspension is then released into the hapa to feed the fry. Swim-up-fry quickly consume yolk particles. Jumping-fry of mahseer attain the fingerling stage after about six months and measure 60-90 mm. They are voracious feeders and are highly photosensitive.

Murrels/Snakeheads

Fishes belonging to family Channidae are generally known as murrels and/or snakeheads. They are air breathing freshwater fishes and are distributed in tropical Africa and south Asia e.g. India, Afghanistan, Bangladesh, China, Malayan Archipelago, Myanmar, Nepal, Sri Lanka, Thailand and Pakistan. These are represented by a single genus *Channa* and 14 species.

Four, *Channa* species i.e. *Channagachua*, *C. marulius*, *C. punctatus* and *C. striata* has been reported from Pakistan. However, *Channa marulius* and *Chana punctatus* are considered to be very valuable as food.

Channa marulius (Saul)

These fish have elongated bodies that are cylindrical from anterior side with scales; the head has plate-like scales. Teeth are present on the jaws and the mouth is fairly large. A single long spineless dorsal and an anal fin are present. Scales are small, cycloid or ctenoid.

Spawning season varies by species. Spawning occurs primarily in summer months (June through August). Most snakeheads build nests by cleaning a circular area in aquatic vegetation. *C. punctata* prepares tunnels leading to the nest column.

Eggs are buoyant due to one or more large oil droplets in the yolk and rise to the surface where they are vigorously guarded by one or both parents. Parental care is a behavioral characteristic of snakeheads. Low fecundity is a general rule among mouth brooding fishes. Fecundity increases greatly in large snakeheads ranging from 2,300-26,000 (Quyyum & Qasim, 1962). Forty thousand oocytes have been reported in *C. marulius* (Jhingran, 1984). Oocytes are small on release by female and range from about 1.0 to slightly over 2.0 mm. Fertilization takes place by male releasing milt (sperm) on oocyte. Development time to hatching varies with water temperature and, to a lesser extent, with species involved. Hatching of eggs takes more than 54 hr at 16-26°C and about 30 hr at 28-33°C (Kahn, 1924).

Following yolk sac absorption, snakehead fry begin feeding on zooplankton. Fry typically remain in shoals until they reach the early juvenile stage, guarded by one or both adult. As larval snakeheads mature to early juvenile stages, the diet changes to small crustaceans and insects, particularly insect larvae. In snakeheads, fry mostly feed on zooplankton following yolk sac absorption. Fry of *C. punctata* feed on phytoplankton. Juveniles feed on insect larvae, small crustacean and fry of other fishes. The adult snakeheads are predators on other fish species, though they do not spare even crustaceans and frogs. Snakeheads have a highly developed air breathing apparatus that enables them to migrate overland by wriggling movements.

Tilapia

Tilapia are native to Africa, but have been introduced in 140 countries of the world including Pakistan. They are disease-resistant, reproduce easily, eat a wide variety of foods and tolerate poor water quality with low dissolved oxygen levels. They are mostly grown in ponds, cages and rice fields in various parts of the world. Total world production of farmed Tilapia was 4,507,000 million MT in 2012 (FAO 2014). Tilapias are rapidly becoming more acceptable worldwide by middle-class and upscale producers.

In Pakistan, fish culture is developing to produce more protein of animal origin. Traditionally, polyculture of Indian major carps and Chinese carps is being done in the country. Pakistan is located north of the Tropic of Cancer and has arid and semiarid climatic conditions with low and irregular rainfall. Much of the land and groundwater of the country is affected with high levels of salinity. Due to these high levels of salinity, the traditionally cultured fish species remain under stress and cannot perform well. As a result, the industry has shown little growth and low productivity. These conditions are responsible for low profitability of fish farming in Pakistan. Tilapia is a good option under such a scenario due to its wide range of tolerance towards salinity and other environmental factors. The omnivorous feeding habit of Tilapia enables it to be cultured in a variety of systems. The commercially important species of tilapia so far introduced in Pakistan are;

- Nile tilapia *Oreochromis niloticus*
- Blue tilapia *Oreochromis aureus*
- Mossambique/Java tilapia *Oreochromis mossambicus*

One of the many advantages of tilapia for aquaculture is that, like the carps, they feed in a lower trophic level. The members of the genus *Oreochromis* are all omnivores, naturally feeding on algae, aquatic plants, small invertebrates, detrital material and the associated bacterial films. In extensive aquaculture, the fish is able to grow by eating algae and detrital matter and the farmer can grow more fish in a given area because the fish are depending directly on the primary productivity of the body of water.

Picture. Tilapia harvested at feeding demonstration in 2013 under FEEDing Pakistan project. Photo courtesy: R.S.N. Janjua, SoyPak (ASA/WISHH)

In intensive systems, Tilapia has the advantage that they can be fed prepared soy based feeds with inclusion of some other plant protein sources. Carnivorous fishes on the other hand, require fish meal or other animal proteins in their diets, making it more expensive than plant protein based feeds. Tilapia exhibit their best growth rates when they are fed a balanced diet that provides a proper mix of protein, carbohydrates, lipids, vitamins, mineral and fiber. Fry and fingerling fish require a diet higher in protein, lipids, vitamins and minerals and lower in carbohydrates as they are developing muscle, internal organs and bone along with rapid growth. Sub-adult fish need more calories from fat and carbohydrates for basal metabolism, hence, lesser protein is required. Adult fish need even less protein.

Although having many good characteristics, the major drawback to tilapia species is the tendency to breed early and overpopulate a pond. Under optimal conditions, some species of tilapia mature at an early age and small size. Each mature female may produce a few hundred to a few thousand offspring every 28 to 45 days. The fast reproductive cycles overpopulate rearing ponds. This over population results in competition for food and little growth, making it difficult to obtain fish above 150 g. The best solution of the problem is to practice mono-sex culture, preferably “All Male Culture of Tilapia”.

Female

Male

Fig. Sexual differentiation of male and females (*Oreochromis niloticus)*

Farming with all male tilapia can be done through three different techniques.

a) Manual separation of sexes, or "hand-sexing", can be performed by visually inspecting the genital papilla of tilapia fingerlings.

An experienced field hand generally achieves at least 95% accuracy. Advantages of "hand-sexing" are that no steroids are required and the constraints of hybridization are eliminated. The technique is commercially feasible for small- and medium-scale operations.

b) Sex reversal is widely done to produce all male progeny in tilapia throughout the world. Sex reversal is the process of administering male steroid hormones to recently hatched fry so that the undifferentiated gonadal tissue of genetic females develops into testicular tissue, producing individuals that grow and function reproductively as males. The procedure must be initiated before the primal gonadal tissue starts to differentiate into ovarian tissue which, at average water temperatures of 24° to 28°C, occurs in *Oreochromis* spp at a size and age of only 11 to 13 mm and 3 to 4 days after hatching. The hormone, 17 alpha-methyl testosterone (MT), is used at 60 ppm in feed for 28 days for complete sex reversal.

c) Another method to control this problem is production of YY Supermales. In this variation, juvenile fish are fed estrogen at a similar dose for a similar time. The intent is to develop an all-female population. Then the functional females, but still genetic males, are mated to normal males. The progeny will then be one-quarter normal females, one-half normal males, and one-quarter will have two male chromosomes, referred as Supermales. When a Supermale, with two Y (male) chromosomes, is mated with a normal female (XX), the progeny should be all XY normal males.

d) Among the commercial tilapia species, a few hybrid crosses produce a higher percentage of male offspring. Crosses between *O. mossambicus* females with *O. urolepis-hornorum* males and *O. niloticus* females and *O. urolepis-hornorum* males yield a higher percentage of male offspring. Crosses between *O. niloticus* females and *O. aureus* males and *O. mossambicus* females and *O. aureus* males can produce up to 85-90% males.

An important advantage of hybridization versus sex reversal is that artificial steroids are not required. However, hybridization is now seldom used for large-scale production of all-male tilapia fingerlings as it has many drawbacks including the need to maintain multiple broodlines of hard to identify different species and need for very careful record-keeping.

Catfishes

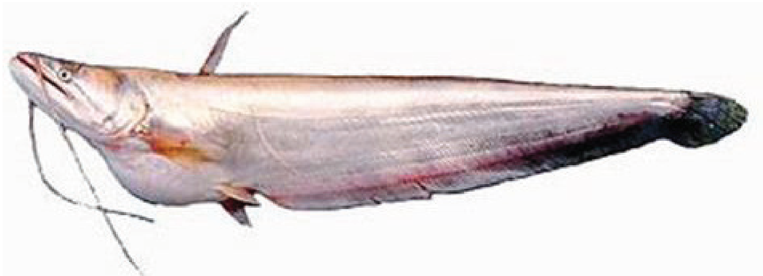
In Pakistan, there is a high demand for carnivorous fish species including catfishes in the market. However, farming of catfishes on a semi-intensive or intensive basis is still not practiced. A major bottle-neck in their farming is lack of appropriate feed, provision of seed and lack of technical information regarding their culture. Keeping in view the market demand of catfishes, the following species may be potential candidates for culture:

Mystus singhala (Singharee)

This fish is found in rivers, canals, ditches, inundated fields and other freshwater areas of Asia: Afghanistan, Pakistan, India, Nepal and Bangladesh. It is a carnivore in the wild. Breeding occurs before the commencement of monsoons. It is an oviparous fish and distinct pair mating, possibly like other members of the same genus, is observed especially in breeding season. Body is elongate and compressed; snout is broad and spatulate. Color is brownish-gray on back, silvery on flanks and belly. A dark well-defined spot is on the adipose dorsal fin.

Malli, *Wallago attu*

This fish is commonly distributed in freshwaters of the sub-continent. It can grow up to 240 cm. Its head is broad and snout depressed. Body is elongated and strongly compressed. Mouth is very deeply clefted, reaching far behind the eyes. Teeth in jaws are set in wide bands. It has two pairs of barbels. Maxillary barbels extend to the anterior margin of the anal fin. Eyes are small with a free orbital margin. Dorsal fin is small, however, anal fin is very long. This fish is found in most parts of Asia ranging from Pakistan to Vietnam and Indonesia and can be found in large rivers, lakes and impoundments.



*Wallago attu (*Malli)

It is a voracious and predatory catfish. Malli is sluggish and hides in holes in river banks and canals associated with deep, still or slow-flowing water with a mud or silt substrate. It stays on the muddy or silty bottom in search of food. Juveniles feed mainly on insects; adults feed on smaller fish, crustaceans, and mollusks. It is an oviparous fish and distinct pairing is observed in pre-monsoon summer. It is highly destructive to other valuable food-fishes. It bites strongly, if handled, with its huge mouth, formidable jaws, and band of conical teeth.

American Channel catfish *Ictalurus punctatus*

This fish is not native to Pakistan. Due to its wide ranges of culture adaptability it is being successfully cultured in the world. Recently this fish was imported into Pakistan and efforts are being made to introduce it in local culture systems.

Ictalurus punctatus (Chanel catfish)

It is usually bluish olive, grey or black on the upper part of the body, becoming white below; dark spots are usually scattered along the sides; older males are dark in color, the head is very wide when seen from the top; long barbels surround the mouth and the tail is deeply forked. This fish inhabits rivers and streams and prefers clean, well oxygenated water. Channel catfish feed primarily on small fish, crustaceans (e.g. crayfish), clams and snails, as well as feeding on aquatic insects and small mammals.

Pangasius bocourti* and *P. hypophthalmus

The Pangasius catfish from South Vietnam has the largest production of any catfish in aquaculture with production of 1,300,000 MT per year. Farms have now been started all across South and Southeast Asia. Pangasius grow well in ponds or cages and can be fed a grain based diet with minimal amounts of fish meal or meat by-products. Pangasius are "air breathers" with a capability to intake air by gulping at the surface and extract oxygen. Pangasius are processed in large amounts in VietNam and are marketed as frozen fillets in many Pakistani grocery stores. Domestic production could provide a significant volume of high quality fish at a good price.

Pangasius hypophthalmus

References

Food and Agriculture Organization, 2014. Global Aquaculture Production Volume and Value Statistics Database.

Jhingran, A. G., 1984. The fish genetic resources of India. Indian Council of Agricultural Research, Bureau of Fish Genetic Resources.

Kahn, H., 1924. Observations on the breeding habits of some freshwater fishes in the Punjab. Bombay Natural History Society Journal 29:958-962.

Mirza, Z.S., Mirza, M.R., Nadeem, M.S., and Sulehria, A.Q.K., 2013. Revised checklist of fishes of Mangla Reservoir, Pakistan. Biologia (Pakistan), 59(1), 1-6.

Mirza, M. R., 2002. Checklist of freshwater fishes of Pakistan. Pakistan Journal of Zoology, suppl. Ser. No. 3: 1-30.

Mirza, M.R. 1990. Fishes. Pakistan Journal of Zoology, 23(4), 339-341.

Mirza, M.R., 2004. Freshwater fishes of Pakistan. 2nd Ed. Urdu Science Board, Lahore, Pakistan. Nelson, 2006. Fishes of the World.

Qasim, S. Z., and Qayyum, A., 1962. Spawning frequencies and breeding seasons of some freshwater fishes with special reference to those occurring in the plains of Northern India. Indian Journal of Fisheries, 8(1), 24-43.

Zafar Iqbal, K. P., and Javed, M. N., 2013. Population dynamics of *Tor macrolepis* (Teleostei: Cyprinidae) and other fishes of Attock Region, Pakistan. Canadian Journal of Pure and Applied Sciences, 2195.

CHAPTER – IV

FISH NUTRITION

Author
Prof. Dr. Muhamad Ashraf
Dean, Faculty of Fisheries and Wildlife
University of Animal and Veterinary Sciences (UVAS)
Pattoki Campus, Punjab - Pakistan.
E-mail: muhammad.ashraf@uvas.edu.pk

Co-authors
Dr. R.S.N. Janjua
Country Representative,
American Soybean Association
World Initiative of Soy in Human Health
Karachi - Pakistan.
E-mail: janjua@soypak.org

Erfan Shahkar
Ph.D. Scholar
Department of Marine Bio-Materials and Aquaculture
Feeds and Foods Nutrition Research Center,
Pukyong National University
Busan 608-737, Republic of Korea.
E-mail: erfanshahkar@yahoo.com

This chapter provides an overview to fish nutrition with a focus on what farmers and feed millers should know about how to prepare nutritious feeds for their fish. The following chapters go into additional details on actual manufacturing of diets, feed practices and the best management practices for the handling and distribution of feed on farm. We are providing an additional chapter on the use of soybean products in aquaculture feeds in recognition of the fact that soybean products are rapidly being substituted for fish meal and fish oils in aquaculture feeds around the world.

INTRODUCTION

Similar to livestock and poultry production, nutrition plays a key role in aquaculture by influencing fish growth, health, product quality, and waste generation. Quality nutrition in fish production systems is essential to economically produce a high quality product. Feed costs often account for over 50% of variable costs of an aquaculture enterprise and thus significantly influence economic returns. In intensive fish farming, feed is even more critical because it represents more than 60-70% of the production costs. Fish nutrition has advanced dramatically in recent years with the development of nutritious, efficiently delivered, and cost-effective commercial diets dependent on knowing a species' nutritional requirements and meeting those requirements with balanced diet formulations and appropriate feeding practices. The development of new species-specific diet formulations supports the aquaculture industry as it expands to satisfy increasing demand for affordable, safe, and high-quality fish and seafood products (Gatlin, 2010). Aquatic animal nutrition is a relatively new science.

MAJOR NUTRIENT GROUPS (MACRONUTRIENTS)

Energy-Yielding Nutrients

Nutrients are required by animals to sustain life processes and allow activity, growth, physiological function and reproduction. Nutrients serve as precursors for the biosynthesis of structural or storage molecules, enzymes, metabolic intermediates, and a plethora of other molecules. A proportion of the nutrients consumed is catabolized to harness chemical (free) energy, which is required or used in anabolic and other life-sustaining processes (Blaxter, 1989; Mayes, 2000). Animals do not simply metabolize energy *per se* instead they metabolize specific nutrients, each with specific roles and metabolic fates (Van Milgen, 2002). Several nutrients or metabolic intermediates derived from nutrients are used simultaneously in the same process, and interactions between nutrients are numerous. A large number of endogenous (genetic, sex, physiological state, nutritional history etc.) and exogenous (stressors such as temperature and photoperiod) factors also affect the fate of nutrients in animals (Blaxter, 1989).

Prepared or formulated diets may be either complete or supplemental. Complete diets supply all the ingredients (proteins, lipids, carbohydrates, vitamins, and minerals) necessary for the optimal growth and health of the fish. Most fish farmers use complete diets, those containing all the required protein (18-50%), lipid (10-25%), carbohydrate (15-20%), ash (<8.5%), phosphorus (<1.5%), water (<10%), and trace amounts of vitamins, and minerals. When fish are reared in high density indoor systems or confined in cages and cannot forage freely on natural feeds, they must be provided a complete diet. In contrast, supplemental (incomplete, partial) diets are intended only to help support the natural food (insects, algae, small fish) normally available to fish in ponds or outdoor raceways. Supplemental diets do not contain a full complement of vitamins or minerals, but are used to help fortify the naturally available diet with extra protein, carbohydrate and/or lipid. Fish, especially when reared in high densities, require a high-quality, nutritionally complete, balanced diet to grow rapidly and remain healthy.

Proteins, lipids and carbohydrates are distinct nutrient groups that the body metabolizes to produce the energy it needs for numerous physiological processes and physical activities. There is considerable variation in the ability of fish species to use the energy-yielding nutrients. This variation is associated with their natural feeding habits, which are classified as herbivorous, omnivorous or carnivorous. Thus, there is a relationship between natural feeding habits and dietary protein requirements. Herbivorous and omnivorous species require less dietary protein than some carnivorous species (NRC, 2011). Carnivorous species are very efficient at using dietary protein and lipid for energy but less efficient at using dietary carbohydrates. The efficient use of protein for energy is largely attributed to the way in which ammonia from de-aminated protein is excreted via the gills with limited energy expenditure. The carnivorous species eat foods with little carbohydrate, and in general they use this nutrient less efficiently. In terms of energy density, proteins, lipids and carbohydrates have average caloric values of 5.65, 9.45 and 4.15 kilocalories per gram ($kcal.g^{-1}$), respectively.

PROTEINS AND AMINO ACIDS

Proteins and amino acids are critical molecules because of the role they play in the structure and metabolism of all living organisms. Proteins and their building blocks, amino acids, are organic compounds that are essential components of all living organisms. Amino acids can link together by a covalent peptide bond between the α-carboxyl end of one amino acid and the α-amino end of other (Brody, 1999).

Proteins are composed of carbon (50%), nitrogen (16%), oxygen (21.5%), and hydrogen (6.5%). They provide the proper ratio of amino acids and are necessary for building of muscle, connective tissues, blood, enzymes, hormones, etc. They are formed by linkages of individual amino acids. Although over 200 amino acids occur in nature, only about 22 amino acids are common. Of these, 10 are essential (indispensable) amino acids (Table 1) that cannot be synthesized by fish and must be provided in diet; these are methionine, arginine, threonine, tryptophan, histidine, isoleucine, lysine, leucine, valine and phenylalanine. Of these, lysine and methionine are often the first limiting amino acids (Table 1). Fish feeds prepared with plant (soybean meal) protein typically are low in methionine but high in lysine; therefore, extra methionine must be added to soybean-meal based diets in order to promote optimal growth and health. Because protein is the most expensive part of fish feed, it is important to accurately determine the protein requirements for each species and size of cultured fish. It is important to know and match the protein requirements and the amino acid requirements of each fish species reared (Wilson, 2005).

Protein levels in aquaculture feeds generally average 25-50% for marine shrimp, 28-32% for catfish, 32-38% for tilapia, 25-40% for carps, 30-50% for snakehead fish (e.g. *Channa marulius*), and 38-42% for hybrid striped bass. Protein requirements usually are lower for herbivorous fish (plant eating) and omnivorous fish (plant-animal eaters) than they are for carnivorous (flesh-eating) fish, and are higher for fish reared in high density (recirculating aquaculture) than low density (pond aquaculture) systems (Craig and Helfrich, 2002). Protein requirements generally are higher for smaller fish. As fish grow larger, their protein requirements usually decrease. Protein requirements also vary with rearing environment, water temperature and water quality, as well as the genetic composition and feeding rates of the fish. Protein is used for fish growth if adequate levels of lipids and carbohydrates are present in the diet. If not, protein may be used for energy and life support rather than growth. Fish are capable of using a high protein diet, but as much as 65% of the protein may be lost to the environment. Most nitrogen is excreted as ammonia (NH_3) by the gills of fish, and only 10% is lost as solid wastes.

Table 1. Essential and non-essential amino acids

Indispensable (essential)	Dispensable (nonessential)
Arginine	Alanine
Histidine	Asparagine
Isoleucine	Aspartic acid
Leucine	Cystine
Lysine	Glutamic acid
Methionine	Glutamine
Phenylalanine	Glycine
Threonine	Proline
Tryptophan	Serine
Valine	Tyrosine

Accelerated eutrophication (nutrient enrichment) of surface waters due to excess nitrogen from fish farm effluents is a major water quality concern of fish farmers. Effective feeding and waste management practices are essential to protect downstream water quality.

LIPIDS AND FATTY ACIDS

Lipids are a heterogeneous group of compounds including fats, oils, steroids, wax and related compounds, which are related more by their physical than by their chemical properties. Fatty acids are a sub-group of lipids which are straight chain hydrocarbons possessing a carboxyl (COOH) group at one end. Lipids are organic substances with some being relatively insoluble in water and soluble in organic solvents (alcohol, ether etc.), and utilized by the living cells. Fatty acid nomenclature includes the number of carbon atoms in the backbone chain, the number of double bonds between the carbons, and the location of the first of the double bonds. The carbon next to the carboxyl group is known as α (alpha), the next carbon β (beta), while the last position is labelled as a "ω" (omega).

Lipids serve many important functions in the animal body such as:

- An excellent source of energy for the body. When oxidized, 1g of fat gives 9 kilocalories of energy.
- Provide essential fatty acids.
- Most of the energy stored in the body is in the form of lipids (triglycerides).
- Phospholipids are important constituent of cell membrane.
- The steroid hormones are critical chemical messengers that allow the tissues of the body to communicate with one another. The prostaglandins exert strong biological effects on both the cells that produce them and other cells of the body.
- Dietary lipid serves as a carrier of the fat-soluble vitamins.
- Lipids protect many vital organs from outer shocks.
- Subcutaneous lipid serves to insulate the body from extremes of cold temperatures.

Lipids are broadly classified into simple, complex and derived, which are further subdivided into different groups.

1. Simple lipids: these are esters of fatty acids with various alcohols.

(a) Fats and oils: esters of fatty acids with glycerol. The difference between fat and oil is only physical; therefore, oil is a liquid while fat is a solid at room temperature.

(b) Waxes: esters of fatty acids (usually long chain) with alcohols other than glycerol. These alcohols may be aliphatic or alicyclic. Acetyl alcohol is most commonly found in waxes.

2. Compound/complex lipids: esters of fatty acids containing groups in addition to an alcohol and a fatty acid.

(a) Phospholipids: lipids containing, in addition to fatty acids and an alcohol, a phosphoric acid residue. They frequently have nitrogen containing bases and other substituents, e.g. in 'glycerophospholipids' the alcohol is glycerol and in 'sphingo - phospholipids' the alcohol is sphingosine.

(b) Glycolipids: lipids containing a fatty acid, sphingosine, carbohydrate and nitrogenous base. Glycerol and phosphate are absent e.g. cerebrosides, gangliosides.

(c) Lipoproteins: macromolecular complexes of lipids with proteins.

(d) Other complex lipids: lipids such as sulfolipids and amino lipids.

3. Precursor and derived lipids: these include fatty acids, glycerol, steroids, other alcohols, fatty aldehydes, ketone bodies, hydrocarbons, fat-soluble vitamins and hormones.

Fatty acids are merely carboxylic acids with long hydrocarbon chains. The hydrocarbon chain length may vary from 10-30 carbons (most usual is 12-18).

The non-polar hydrocarbon alkane chain is an important counter balance to the polar acid functional group. In acids with only a few carbons, the acid functional group dominates and gives the whole molecule a polar character. However, in fatty acids, the non-polar hydrocarbon chain gives the molecule a non- polar character. The most common fatty acids are listed in Table 2. Note that there are two groups of fatty acids:

Saturated fatty acid

- No double bonds.
- Solid at room temperature.
- Implicated in coronary heart disease.

Unsaturated fatty acid

- Mono unsaturated fatty acid, poly-unsaturated fatty acid (PUFA) and long chain poly unsaturated fatty acid (LC-PUFA).

- Has double bonds
- Liquid at room temperature
- Less stable, prone to rancidity

A fatty acid that cannot be synthesized in the body is called an essential fatty acid (EFA). For instance, linoleic acid and α-linolenic acid, which are the primary precursor molecules for the (n-6) and (n-3) family of fatty acids in animal tissues, must come from the diet. Dietary deficiency of these essential fatty acids results in various pathologies, the animal ceasing its growth and reproduction, and eventually death (Das, 2006). The biologically active Poly-Unsaturated Fatty Acids (PUFAs) required for many essential metabolic and physiological processes are the LC-PUFA, 20:4n-6 (ARA, Arachidonic acid), 20:5n-3 (EPA) and 22:6n-3 (DHA) (Das, 2006). In contrast the shorter chain C_{18} PUFA, typified by linoleic acid 18:2n-6 and α-linolenic acid 18:3n-3, have no specific metabolic roles in themselves, although they can serve as precursors for the corresponding n-6 and n-3 LC-PUFA (Sargent *et al*. 1995). Species vary in their capacity to convert C_{18} PUFA to LC-PUFA. In species that cannot perform these conversions, dietary C_{20} and C_{22} LC-PUFA are essential, and their C_{18} homologues do not satisfy EFA requirements. In species that can perform the conversions, C_{18} PUFA, and C_{20}-$_{22}$ LC-PUFA can all be termed EFA with the LC-PUFA often being more effective nutritionally than their C_{18} counterparts. Note that vertebrates and crustaceans are unable to interconvert the n-6 and n-3 PUFA families (Fig.1).

Reported estimates for juvenile and sub-adults of freshwater and diadromous fish species indicate that EFA requirements can be satisfied by the C_{18} PUFA, 18:3 n-3, and 18:2 n-6, at around 1% of the diet dry weight. In terms of EFA, freshwater/diadromous species were traditionally subdivided into three groups: Coldwater species including salmonids that have a higher requirement for α-linolenic acid (18:3n-3), warm-water species (e.g. tilapia) that have a higher requirement for linoleic acid (18:2n-6), and species that require significant amounts of both e.g. common carp. Therefore, it is likely that all freshwater and diadromous fish require both n-3 and n-6 PUFA. There is evidence that n-3 LC-PUFA and DHA may be more important and, possibly, essential in larvae of some species of freshwater fish compared to adults or juveniles (Webster and Lovell 1990; Wirth *et al*. 1997).

Dietary lipids have been shown to have sparing effect on the utilization of dietary protein. For example, the level of protein in the diet of Nile tilapia can be reduced from 33.2 to 25.7% by increasing dietary lipid from 5.7 to 9.4%, and carbohydrate from 31.9 to 36.9% (Li *et al*. 1991). Research evaluating dietary lipid sources showed that Nile tilapia that were fed diets supplemented with soybean or corn oil rich in linoleic acid (18:2n-6) showed better growth performance than those that were fed diets containing fish oil rich in 20:5n-3 PUFA and beef tallow rich in 18:1n-9 (Takeuchi *et al*. 1983). Dietary fish oil is superior to corn oil in promoting growth of rainbow trout and the yellow-tail species. Dietary α-linolenic acid (18:3 n-3) gives a positive growth response for rainbow trout which may be attributed to a dietary requirement for n-3 fatty acids.

Table 2. Classification of important fatty acids based on number of carbons in the chain, number of double bonds, and location of the first of the double bonds.

Names	Number of C atoms, number of double bonds and location of first double bond	Melting point ^{0}C	Sources
Saturated Fatty acids			
Lauric acid	12:0	44	
Myristic acid	14:0	58	Butter, Animal Fat
Palmitic acid	16:0	63	
Stearic acid	18:0	70	Animal Fat
Un-Saturated Fatty acids			
Oleic acid	18:1n-9	16	Plant, Animal Fat
Linoleic acid	18:2n-6	-5	Plant, Animal Fat
α-linolenic acid	18:3n-3	-11	Fish
Arachidonic acid	20:4n-6	-50	Fish
EPA (eicosapentaenoic acid)	20:5n-3	23	Algae and Fish
DHA (docosahexaenoic acid)	22:6n-3	23	Algae and Fish

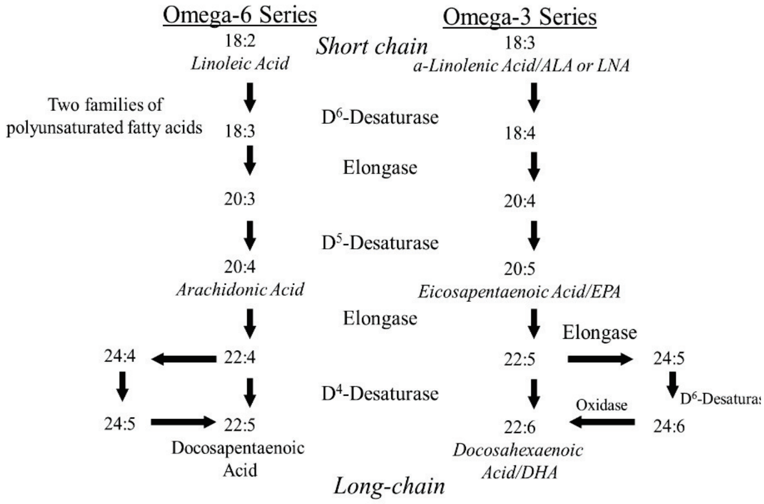


Fig.1. Pathway of biosynthesis of C_{20} and C_{22} long chain poly unsaturated fatty acids (LC-PUFA) from n-3, n-6, and n-9 C_{18} PUFA. (Adapted from Simopoulos, 1991).

CARBOHYDRATES

Carbohydrates (starches and sugars) are the most economical and inexpensive sources of energy for fish diets. Although not essential, carbohydrates are included in aquaculture diets to reduce feed costs and for their binding activity during feed manufacturing.

Carbohydrates are the main source of energy in most animal diets and are classified based on the constituent sugars, structure, composition, degree of polymerisation and glycosidic linkage into *e.g.* non-monomer carbohydrates such as oligosaccharides (lactose, maltose), polysaccharides (starch, chitin, cellulose) and monomer sugars (glucose, fructose) (Englyst & Hudson, 1996). Carbohydrate properties, such as digestion and absorption rate, viscosity, structural features, water-binding capacity and fermentation ability in the GI tract, are of vital importance for their nutritional effects (Asp, 1996).

In fish, carbohydrates are stored as glycogen that can be mobilized to satisfy energy demands. They are a major energy source for mammals, but are not used efficiently by fish (Craig and Helfrich, 2002). For example, mammals can extract about 4 kcal of energy from 1 gram of carbohydrate, whereas fish can only extract about 1.6 kcal from the same amount of carbohydrate. Up to about 20-35% of dietary carbohydrates can be used by fish. Fiber, another category of carbohydrates, is not really a nutrient and fish hardly require it. When fiber is high, digestibility of the feed is decreased. Excess fiber will increase pollution of the pond. Less than 4% of fiber content in the diet is recommended for fish.

Dietary starches are useful in the extrusion manufacture of floating feeds. Cooking starch during the extrusion process makes it more biologically available to fish. However, using the appropriate level of carbohydrates in aqua feeds is of great importance, because if the appropriate amount of carbohydrates is not provided, this may have negative effects on nutrient utilization, growth, metabolism and health (Li *et al.*, 2012; Erfanullah & Jafri, 1998).

MICRONUTRIENTS

Micro-nutrients cover both vitamins and minerals which are essential dietary element that are needed only in very small quantity to play a pivotal role in all the metabolic pathways going on in fish.

VITAMINS

Vitamins are organic compounds that are necessarily required in trace amounts from an exogenous source for normal growth, reproduction, and health of fish. They often are not synthesized by fish, and must be supplied in the diet. Vitamins are classified in i) water-soluble and ii) fat-soluble. Water-soluble vitamins include those in the B complex and C. Eight of the water-soluble vitamins are required in relatively small amounts, have primarily coenzyme functions, and are known as vitamin B complex. Three of the water-soluble vitamins, choline, inositol, and ascorbic acid (vitamin C), are required in larger quantities and have functions other than coenzymes. Of these, vitamin C probably is the most important because it is a powerful antioxidant and helps the immune system in fish. Yet, it is not always possible to correlate a singe of deficiency with a diminished function for an enzyme system for which that vitamins is essential. For some warm water fish, intestinal synthesis by microorganisms supplies the requirement of certain vitamins. Thus, deficiency signs result only in those cases when antibiotics are fed along with a deficient diet. A constant supply of essential water-soluble vitamins is required to prevent deficiency signs in fish, because these vitamins are not stored in body tissues (Table 3; Fig. 2).

The **fat-soluble** vitamins dissolve in lipids which include vitamin A (retinol), vitamin D (cholecalciferol, (vitamin E (tocopherol) and vitamin K (phylloquinone) (Table 3). Of these, vitamin E receives the most attention for its important role as an antioxidant (Halver, 2005). The fat-soluble vitamins (Table 3; Fig. 2) are absorbed in the intestine along with dietary lipids; therefore, conditions favorable for lipid absorption also enhance the absorption of fat-soluble vitamins. As fish seem to lack a lymphatic system as found in mammals, lipid and fat-soluble vitamins most likely are transported to the peripheral tissues via the portal vein and the liver.

Deficiency of each vitamin has certain specific symptoms, but reduced growth is the most common symptom of any vitamin deficiency. Scoliosis (bent backbone symptom) and dark coloration may result from deficiencies of ascorbic acid and folic acid vitamins, respectively. Animals store fat-soluble vitamins, either actively in specific cell compartments, if dietary intake exceeds metabolic needs. Thus, animals can accumulate enough fat-soluble vitamins in their tissues to produce a toxic condition (hypervitaminosis).

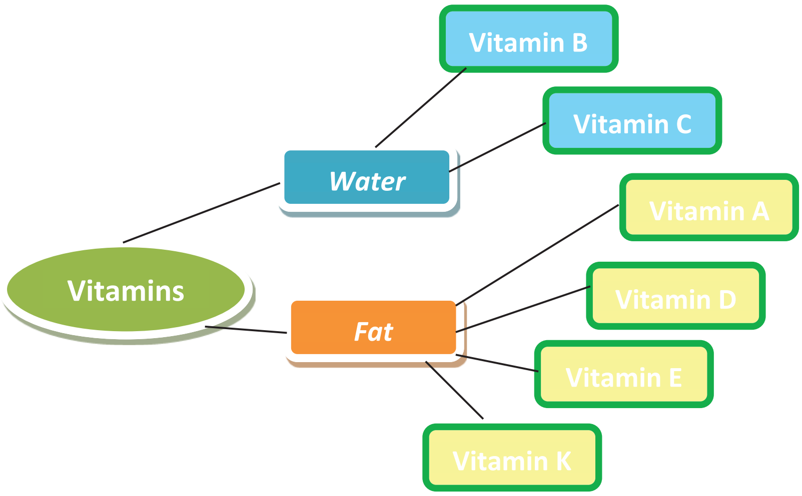


Fig. 2. Water soluble and fat soluble vitamins

Table 3. Vitamins and some of their major functions in fish

Fat-soluble vitamins	**Function**
Vitamin A, Retinol	Epithelial tissue maintenance, vision
Vitamin D, Cholecalciferol	Bone calcification, parathyroid hormone
Vitamin E, Tocopherol	Biological antioxidant
Vitamin K, Phylloquinone	Blood clotting, skin integrity
Water-soluble vitamins	**Function**
Vitamin B_1, Thiamin,	Carbohydrate metabolism
Vitamin B_2, Riboflavin,	Hydrogen transfer
Vitamin B_3, Niacin	Hydrogen transfer
Vitamin B_5, Pantothenic Acid	Lipid and carbohydrate metabolism
Vitamin B_6, Pyridoxine	Protein metabolism
Vitamin B_7, Biotin	Carboxylation and decarboxylation
Vitamin B_9, Folic Acid	Single-carbon metabolism
Vitamin B_{12}, Cyanocobalamin,	Red blood cell formation
Choline	Lipotrophic factor, component of cell membranes
Inositol	Component of cell membranes
Vitamin C, Ascorbic Acid	Blood clotting, collagen synthesis

MINERALS

Information concerning mineral nutrition of fish and crustaceans is limited compared to most of other nutrients groups. In addition, there is much less information on mineral requirements of aquatic species compared to terrestrial animals, in part because of complication in that fish can absorbs some minerals from aquatic medium in which they live, in addition to from their diets. Nevertheless, the basic metabolic function of the various mineral elements is the same for aquatic and terrestrial animals with exception of osmo-regulation.

The metabolism of various minerals by aquatic organism is influenced not only by dietary concentrations but also by the concentration and relative composition of dissolved ions in the aquatic medium, because they may influence the organism's osmoregulation, ion regulations, and acid:base balance (Moyle and Cech, 2000). Numerous minerals can be absorbed by the gills and contribute to meeting metabolic requirements. The uptake of minerals from the diet and aquatic medium, and excretion of minerals in the urine and feces are influenced by osmoregulatory processes in response to salinity of the aquatic medium.

Hyperosmotic: Organisms in freshwater are characterized as being **hyperosmotic** to the environment. They continually lose small ions from the gills, and water is passively taken up such that they do not drink water but excrete large quantities of dilute urine (Figure 3.)

Hyposmotic: In contrast, organisms in seawater are **hyposmotic** to the environment and thus lose water (but gain monovalent ions from the environment) such that they must drink water. The excess salts ingested are primarily excreted by specialized chloride cells in the gills and opercular skin epithelia via active transport, whole the kidney excretes primarily divalent ions in small volumes of urines and other salts are concentrated in feces (Figure 3.)

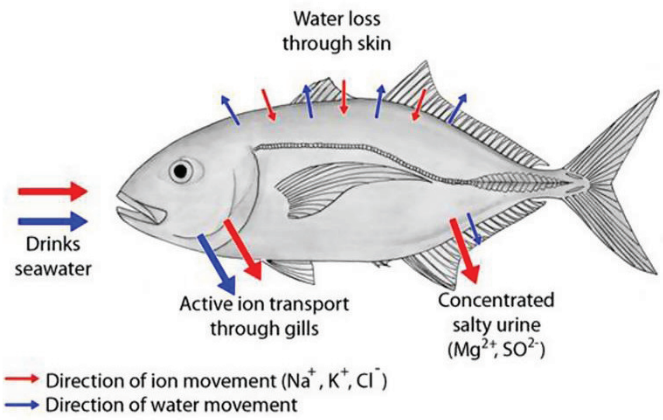


Figure 3. Osmotic relationships in fish, (image from Wikipedia).

Minerals are inorganic elements necessary in the diet for normal body functions. They can be divided into two groups; i) macro-minerals, and ii) micro-minerals, based on the quantity required in the diet and the amount present in fish.

Macro-minerals: Common macro-minerals are sodium, chlorine, potassium, calcium, magnesium, sulfur, and phosphorous (Table 4). The function of macro-minerals, those required in the diet and body at relatively high concentration, include the formation of skeletal structures and other hard tissues (e.g., fin rays, scales, teeth, and exoskeleton), electron transfer, regulation of acid:base equilibrium, the production of membrane potentials, and osmoregulation. These minerals regulate osmotic balance and aid in bone formation and integrity.

Micro-minerals (trace minerals) which are typically required in the diet and body at much lower concentrations than the macro-minerals, are important components of hormone and enzyme system, serves as cofactors and/or activators of a variety of enzymes, as well as participate in a wide variety of biochemical processes. The most commonly recognized trace minerals included chromium, copper, iodine, iron, manganese, selenium and zinc. Fish can absorb many minerals directly from the water through their gills and skin, allowing them to compensate to some extent for mineral deficiencies in their diet.

Both vitamins and minerals have wide variety of functions. They are structural component of hard and soft tissues, cofactors and/or activators of enzymes (Table 4). They serve as osmo-regulators and maintain acid-base balance. They are inevitable for production of membrane potentials and transmission of impulses.

Table 4. Minerals and Some of Their Prominent Functions and Deficiency Signs Observed in Fish and Shrimps

Mineral	Functions	Deficiency Signs
Macro-minerals		
Calcium (Ca)	Skeletal tissues, membrane permeability	Impaired growth and hard issues mineralization
Chlorine (Cl)	Osmotic balance	Impaired growth
Magnesium (Mg)	Enzyme Activator	Tetany, muscle flaccidity
Phosphorus (P)	Skeletal tissues, phospholipids	Impaired growth, reduced hard tissues mineralization, skeletal deformities, fat accumulation
Potassium (K)	Osmotic balance, acid:base equilibrium	Convulsions, tetany
Sodium (Na)	Osmotic balance, acid:base equilibrium	Impaired growth
Micro-minerals		
Copper (Cu)	Metalloenzymes	Impaired growth and reduced activity of copper-containing enzymes
Cobalt (Co)	Vitamin B_{12}	Anemia
Chromium (Cr)	Carbohydrate metabolism	Impaired glucose utilization
Iodine (I)	Thyroid hormones	Thyroid hyperplasia
Iron (Fe)	Hemoglobin	Impaired growth, anemia
Manganese (Mn)	Organic matrix of bone	Impaired growth, skeletal abnormalities, cataracts
Molybdenum (Mo)	Xanthine Oxidase	Reduced enzyme activity
Selenium (Se)	Glutathione peroxidase	Impaired growth, anemia, exudative diathesis, reduced activity of glutathione peroxidase
Zinc (Zn)	Metalloenzymes	Impaired growth, cataracts, skeletal abnormalities, reduced activity of various zinc metalloenzymes

NUTRITIONAL PHYSIOLOGY

Digestion and Metabolism

There are a number of processes that feed undergoes in the body of fish. These include ingestion, digestion, absorption, utilization of ingested food and excretion of waste materials. Digestion is the process of solubilizing and degrading nutrients into smaller components and elements that can transported across the intestinal wall to support physiological processes. Fish can be herbivorous (milkfish, and some carps), detritivorous (grey mullets), carnivorous (salmonids, basses, seabreams, flounders, and groupers) or omnivorous (channel catfish and tilapia). Some have mixed feeding schedules, meaning differences do occur in early and later feeding, for example consuming zooplankton in larval stages and feeding on plant materials during the adult stage. There is lot of variation among digestive systems which correspond to feeding habits of fish. Fish that are constantly grazing on algae, detritus and zooplankton can have a minimal stomach e.g. cyprinids or carp, while piscivores will require a relatively large stomach.

TYPES OF STOMACHS

Looking at the general anatomy of fish, the digestive tract can be seen prominently.

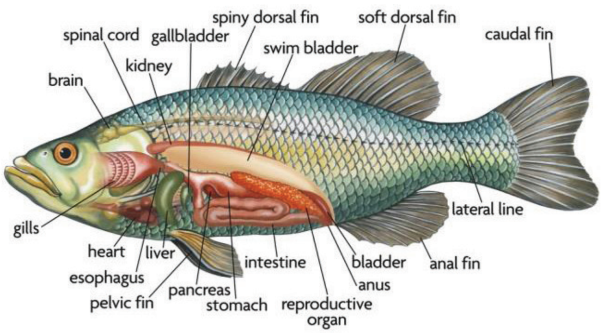


Fig. 4. Typical anatomy of fish (Neuroblog, Stanford University)

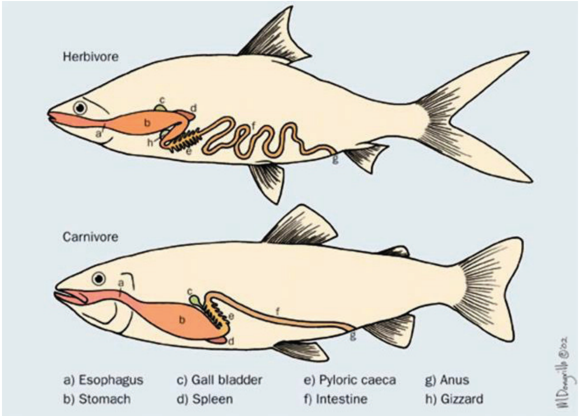


Fig. 5: Comparative Digestive Tracts of Herbivorous and Carnivorous Fishes (Biokids, Univ. of Michigan)

Depending on feeding habits of fish, type and shape of stomach vary and can be compared by relative gut length (Rust, 2005); However, note that all "pure" predators have a stomach and teeth for grasping or tearing. The relationship between the body length and the length of the digestive tract predict the feeding habits of many fish species. This is what is called ***relative gut length*** **(RGL**) which is the ratio between the gut and the body lengths. A high RGL is indicative of species consuming a high proportion of detritus, algae, or plant materials (Figs. 4 and 5).

In carnivores, (rainbow trout or a bass) ***mean relative gut length*** **(mRGL)** in relation to body length can be 0.65-0.8. Omnivores (tilapia, catfishes, and common carp) consuming a mix of animals, plants, detritus and bacteria have gut length to total body length ratios of 1.5 to 4.5. Fully herbivorous fish (e.g. silver carp) which totally depend on vegetable food sources or phytoplankton normally have much longer gut lengths and their ratio to body length can be 15.5.

MORPHOLOGY

Mouth, buccal cavity, and pharynx

Once food is sensed and located, fish must capture it so it can be ingested. Oral or buccal cavity (mouth) is the area where food is first consumed by fish. It extends from the jaw to oesophageal sphincter. The posterior portion of the mouth near the esophagus and gills is called pharynx. Mouth is essentially associated with pre-digestive processing of the food which includes-selection, seizure and orientation. Stomach-less fishes such as carp do not have teeth on the jaw as opposed to predatory fish. Buccal teeth assist in food capture and holding but not in crushing or tearing of prey. Lips are primary feed capturing organs. So structures which aid in capture and retention of food are taste buds, teeth gill rakers (Fig 6), tongue and oesophagus.

Teeth: Fishes which consume macrophytes have anatomical modifications to aid in ingestion and mastication of plant material. Notable adaptations are bi- and tri-cuspid teeth (single projection point or elevation) (e.g. Blue tilapia) which aid in cutting and macerating of macrophytes or strong and specialized pharyngeal teeth with flattened, serrated and rasping surface to cut shred and grind macrophytes (e.g. grass carp). Some species cultured in tropics are filter-feeders, filtering surrounding water for phytoplankton and zooplankton, trapping organisms and ingesting them, paddlefish for example.

Gills: The filtering apparatus is composed of gill rakers, posterior gill arches which are modified to form a fine sieve. The structure and function of gill rakers are parallel to or complementary to the structure and function of teeth in most species. Like teeth, their structure relates to feeding habit of fish. Gill rakers in some species are fine and comb like and are used to strain small particles from the water. While they play a major role in feeding they also protect the delicate gill filaments located on the other side of the gill arches.

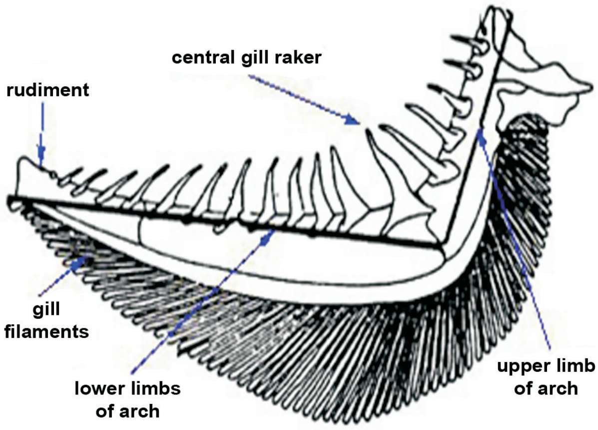
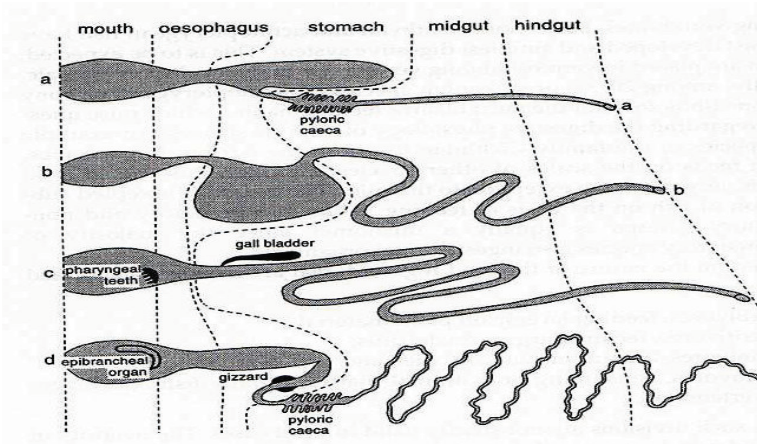


Fig. 6: Structure of gill filaments and rakers.

Digestion: Digestion comprises ingestion of food and its break down into smaller particles for comfortable digestion by digestive enzymes and then its absorption in blood vessels scattered in fish intestine. It is a universal practice that nutrients that fish ingest in prepared feeds are broken down by digestive fluids and enzymes irrespective of the type of species and then absorbed from the gastrointestinal (GI) tract into the blood. The digestion process in fish is similar to that in other monogastric animals; it involves physical, chemical and physiological processes within the GI tract. There is a wide range in the sizes and shapes of GI tracts in fish, but they all generally consist of the same basic structures —the esophagus, acid-producing stomach and intestine (though some fish, such as cyprinids, do not have an acidic stomach). The GI tract also includes pyloric ceca, which are protrusions posterior to the stomach that increase the absorptive area of the GI tract. Accessory organs that interface with the GI tract include the pancreas, which produces a variety of digestive enzymes, and the liver and gall bladder, which produce and store bile salts for emulsification of lipids in the GI tract. Protein digestion begins in the stomach, a low-pH environment resulting from hydrochloric acid secretion and the proteolytic enzyme pepsin. Upon exiting the stomach, the ingesta (chyme) is neutralized by fluids in the intestine and further acted upon by enzymes from the pancreas and intestine. These enzymes aid in the breakdown of complex proteins, carbohydrates and lipids into small molecules that are eventually absorbed into the blood.

Oesophagus: Commonly called gullet, is short, expandable and may not be clearly demarcated from the stomach, or the intestine in stomachless fishes. It is lined with stratified epithelium beneath which is basement membrane. It has few club cells and even taste buds. It acts as well lubricated transit tube for the food adding fluid and mucous during passage into stomach or intestine. Glands and sacs perform functions of food storage, trituration (reducing the particle size of a substance by grinding) and mucous production. Proteolytic activity is normally absent except in *Girella tricuspidata.*

Stomach: Not all fishes have stomach. When present, size of stomach is related to duration between meals and nature of food. Stomach wall consists of number of layers characteristic of vertebrates. Organization of different layers in order from peritoneal cavity to lumen is; Serous membrane (serosa) is a smooth membrane consisting of a thin layer of cells which secrete serous fluid and muscularis is a layer of circular muscles (Fig. 7).



The Digestive System of four fish described in the text, arranged in order of increasing gut length. (a) Rainbow trout (carnivore). (b) Catfish (omnivore emphasizing animal sources of food). (c) Carp (omnivore, emphasizing plant source of food). (d) Milkfish (microphagous planktivore) (From Smith, 1980)

Fig. 7. Digestive systems of different fish species from De Silva and Anderson (1995).

Mucosa is an innermost layer of stomach and consists of submucosa, muscularis mucosae, stratum compactum, lamina propria and mucosal epithelium. Stomach has two regions, anterior and pyloric. Notable differences between two regions are; Presence of gastric glands and prominence of musculature in anterior stomach. Stomach is highly extendable organ e.g. in brown trout enlargement of stomach up to 30-35% in longitudinal direction and up to 75% in diameter may occur.

Intestine: It is simple, columnar absorbing epithelium lined with brush border of microvilli which is typical of absorptive tissues. Microvilli substantially increase surface area of cells. This allows greater contact between cells and nutrients in lumen and provides enhanced cell surface for digestion and nutrient uptake that would otherwise occur with smooth surfaced cell. Other common constituents are goblet cells and cellular migrants (lymphocytes and various types of granulocytes) which in fish with stomachs are zymogen granules. In stomachless fishes this function is performed by goblet cells of intestine which are chiefly mucous producers. Intestinal epithelium also contains pear shaped or rodlet cells function of which is controversial; they are, however, considered stages in life cycle of goblet cells, unicellular glands or even protozoan parasites by different authors. In young fish mucous membrane is mostly smooth, as fish ages longitudinal and zig-zag folds appear, finally leading to sponge like structure. Unlike intestinal length, the intestinal surface area relative to body size tends to decrease with growth. According to physiological functions intestine has two sections. Anterior section where bile and pancreatic juices are received contains large numbers of chylomicrons in epithelial cells. Distal portion of intestine is associated with pinocytotic activity and cells containing granules consisting of absorbed nutrients are present in it.

Rectum: Presence of a rectum varies with species. It is the most distal part of the intestine and usually possess a thicker muscular coat and with a marked increase in number of goblet cells and sometimes granulocytes. A true rectum delineation from intestines by ileorectal valve has been observed only in a very few species. The number of goblet cells in the rectum changes with feeding habits and starvation in fish.

Pyloric Caeca: Also called intestinal caecae, form auxiliary appendages in teleosts, having similar structure to that of intestine. They have well developed muscularis consisting of circular muscles. In some cases epithelial cells of pyloric caecae may have cilia. Presence and absence and number of pyloric caecae vary with species. Though it is not always species specific but in certain instances this feature has been used for taxonomic purposes. In certain cases number of caecae has been correlated with the bulk of food consumed, however, it is unclear. Further, it is also not clear whether number of caecae influences digestibility of individual nutrients or not. Some suggest that pyloric caecae enhance absorption of amino acids, carbohydrates and lipids but others failed to observe such effect on protein and energy. They supplement digestive functions of stomach, increases intestinal surface area, serves as accessory food reservoir, a site of carbohydrate and lipid absorption or site of resorption of water and inorganic ions. They function as space saving device or an endocrine gland precursor of pancreas.

Pancreas: Enzymes are stored as zymogens, proteases produced by intestine convert trypsinogen into trypsin, which in turn activates others. HCO_3- neutralizes HCl entering intestine for alkali digestion. Proteases (trypsin, chymotrypsin, carboxypeptidases and elastase) function at pH 7.0. Trypsin cleaves peptide linkages at carboxyl groups of lysine or arginine. Elastase attacks peptide bonds on elastin. Carboxypeptidases hydrolyses terminal peptide bond of their substrates. Amylases digest carbohydrates at non-acidic pH. Chitinase splits chitin into dimers of N-acetyl-D-glucosamine (NAG) which is further broken down by NAGase. Lipases hydrolyse triglycerides, lipids, phospholipids and wax esters. Bile (secreted by liver) consists of bile salts, organic anions, cholesterol, phospholipids, inorganic ions. Chymotrypsin attacks peptides with carbonyls from aromatic side chains. Bile makes intestinal medium alkaline, and emulsify lipids. Most bile salts are reabsorbed from intestine and returned to liver in enterohepatic circulation. Intestinal enzymes are secreted by brush border of epithelium but could be partly pancreatic in origin. Aminopeptidases (alkaline and acid) split nucleosides. Polynucleotidase split nucleic acids. Lecithinase splits phospholipids into glycerol and fatty acids. Various carbohydrate digesting enzymes digest carbohydrates.

Absorptive processes: Transport of nutrients from intestinal lumen into enterocytes can occur by pinocytosis (for large molecules), simple diffusion, ion exchange or active transport. Absorption of monovalent ions in the intestine is through Na^+/H^+ and Cl^-/HCO_3^- ion exchange system. In active transport two important enzymes via alkaline phosphatase and γ–glutamyltransferase are involved. Both these enzymes are membrane linked and serve as proxy for enhanced nutrient uptake. Alkaline phosphatase helps in lipid and protein absorption while the later helps for amino acid transport. In mammals seven transport systems have been identified for amino acid transport in the brush border of intestinal mucosa.

Post absorptive transport and processing: Nutrients absorbed by the enterocytes are transported to liver via circulatory and lymphatic systems. Liver is central metabolic organ and has primary role in intermediary metabolism and an important role in digestion, detoxification and waste removal. Liver is primary regulating organ for circulating levels of amino acids, lipids and glucose. From enterocytes, proteins are transported to the liver as free amino acids and lipoproteins. Lipids are re-esterified to phospholipids and triacylglycerols in the enterocytes and transported to the liver as lipoprotein complex called chylomicrons, or very low density lipoproteins (VLDL). Carbohydrates are transported as glucose in the blood.

Metabolism

Metabolism is the biological utilization of absorbed nutrients for synthesis (e.g., growth) and energy expenditure. In most aquatic species, the protein sparing effect of carbohydrates is good but its metabolism has a long lag time associated with it. So once a carbohydrate is ingested/digested, blood levels quickly rise, but require extended periods to decline

ENERGY UTILIZATION

Energy – Why does a fish need it?

- Energy is not a food stuff, Formed from breakdown of feed (Protein, lipids and carbohydrates
- Activity
- Chemical reactions
- Nervous system
- Osmoregulation
- Growth
- Energy reserves
- Excretion of wastes
- Fecundity and reproductive performance
- Maintaining position in water

Metabolic Pathways

The liver plays a major role in directing the various nutrients to specific organs and tissues to be metabolized for energy. The same basic metabolic pathways for converting amino acids, carbohydrates and lipid into energy have been observed in fish as in terrestrial animals. It is preferable for dietary carbohydrates or lipid to be metabolized for energy so that protein (amino acids) can be used for tissue synthesis. To ensure this, there must be a proper balance of dietary protein to energy to optimize fish growth and lean tissue accretion. Energy-to-protein ratios ranging from 8 to 10 kcal of DE/g of protein (33 to 42 kJ/g) are optimal for various fish species.

Metabolism starts glycolysis in cytoplasm. Glycolysis ends up in pyruvate an intermediary product which enters in Krebs cycle in Mitochondria. Krebs cycle yields several ATP producing products/molecules which move to Electron Transport Chain where energy (ATP) is produced.

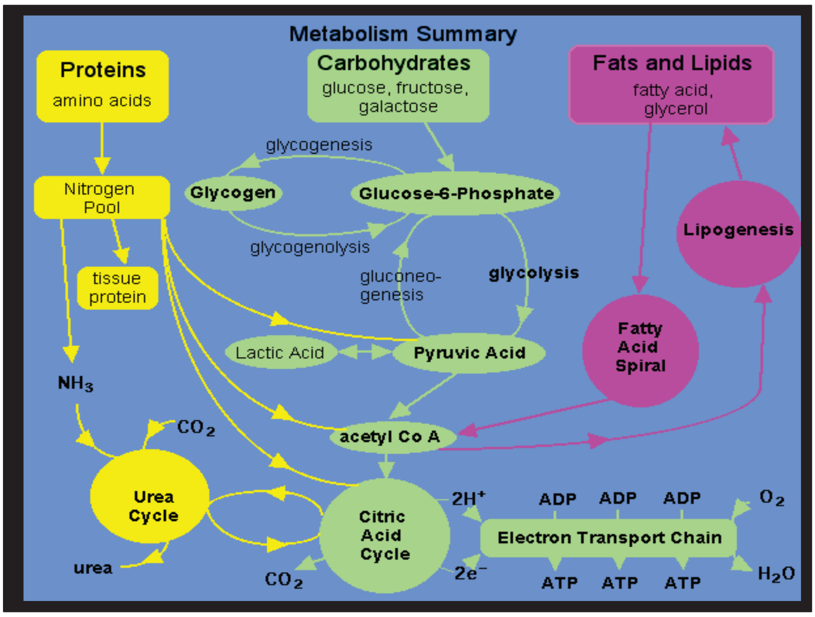


Figure 8. Summary of Metabolic Pathways (from: Virtual Chembook, Elmhurst College, C. Ophardt)

Energy intake is divided among all energy-requiring processes (Fig. 8). The magnitude of each depends on quantity of intake plus animal's ability to digest and utilize that energy and can vary by feeding mode: carnivorous vs. herbivorous. The terms DE= Digestible Energy; ME= Metabolizable energy; Hp= Heat of production; NEp= Net energy of production and NEm= Net energy for maintenance.

Energy content of a substance is typically determined by completely oxidizing (burning) the compound to carbon dioxide, water and other gases. The amount of energy given off is measured and known as gross energy (GE) and measured by a device known as a bomb calorimeter although other devices such as gradient chamber, infra-red detector can also be used.

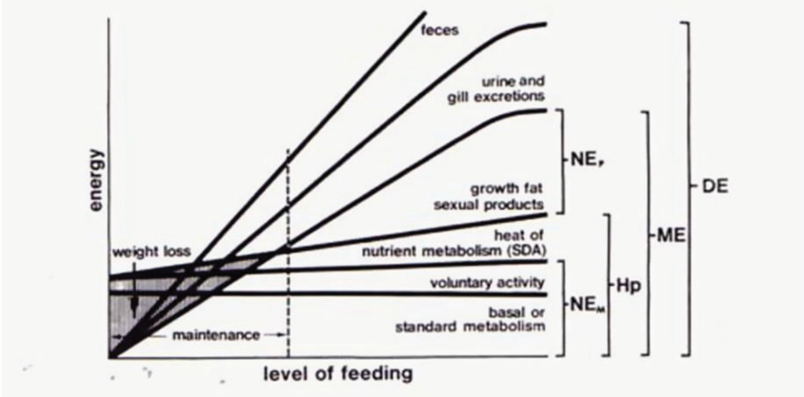


FIG. 1.2

Energy intake and distribution among energy-requiring processes. (From Smith, *In* "Studies on the Energy Metabolism of Cultured Fishes", 1976 Thesis, Cornell University.)

Figure 9. Energy intake and distribution among energy requiring processes.

Energy flows in a fish body

Energy flow as used in the body of fish is often shown as a diagram:

These are described by the terms as follows:

- Gross energy (GE): energy released as heat resulting from combustion (kcal/g)
- Intake Energy (IE): gross energy consumed in food (COH, lipid, protein)
- Fecal Energy (FE): gross energy of feces (undigested feed, metabolic products, gut epithelial cells, digestive enzymes, excretory products)
- Digestible Energy (DE): IE-FE
- Total heat production (HE): energy lost in the form of heat. The heat lost is sourced from metabolism, thus, HE is an estimate of metabolic rate and is measured by temperature

change (calorimetric) or oxygen consumption rate divided into a number of constituents as per energy flow diagram (Fig. 10).

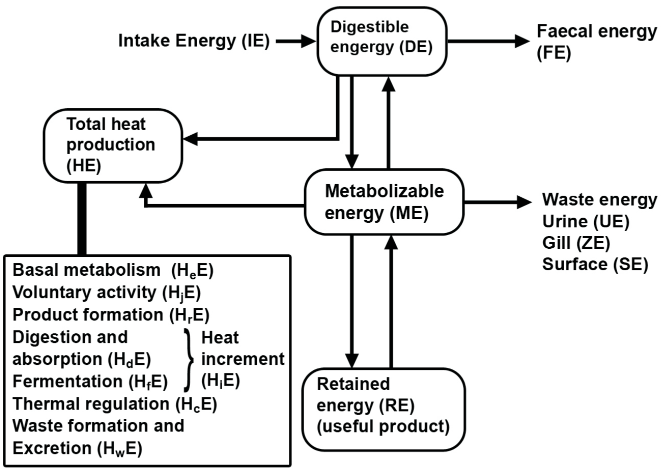


Figure 2.4 Energy flow in an aquatic organism.

Fig.10. Energy flow in the body of fish referred to as bioenergetics model.

Total heat production

- Basic metabolic rate (H_e E_e): heat energy released from cellular activity, respiration, blood circulation, etc.
- Heat of activity (H_i E): heat produced by muscular activity (locomotion, maintaining position in water)
- Heat of thermal regulation (H_cE): heat produced to maintain body temperature (above zone of thermal neutrality)

DIETARY REQUIREMENTS

Feeds and feedstuffs contain variety of nutrients and energy sources essential for fish growth, reproduction and health. Deficiencies of different nutrients in feed can reduce growth rates and/or can lead to variety of nutritional abnormalities and invite other nutritional ailments and infectious diseases due to reduced immunity of fish (National Research Council, 1993). Dietary requirements can be and have been established for energy, proteins and amino acids, lipids, minerals, and vitamins. Depending on feed habits, living environment and stage of fish development and growth, there is lot of variation in nutrient requirements. Due to lot of variations it is hard to stick to one value of the specific nutrient in diet, so ranges of nutrients applicable in formulated feeds for few culturable and commercially important fish species have been provided for the interest and ease of the farmer.

FEED TYPES

Commercial fish diets are manufactured as either extruded (floating or buoyant) or pressure-pelleted (sinking) feeds (Craig and Helfrich, 2002). Both floating and sinking feed can produce satisfactory growth, but some fish species prefer floating, others sinking. Shrimp, for example, prefer a sinking feed, but most domesticated fish species (carps, tilapias, catfishes, salmonids) can be trained to accept a floating pellet. Extruded feeds are more expensive due to the higher manufacturing costs. Usually, it is advantageous to feed a floating (extruded) feed, because the farmer can directly observe the feeding intensity of his fish and adjust feeding rates accordingly. Determining whether feeding rates are too low or too high is important in maximizing fish growth and feed use efficiency. Feed is available in a variety of sizes ranging from fine crumbles for small fish to large (1/2 inch or larger) pellets. The pellet size should be approximately 20-30% of the size of the fish species mouth gape (Gatlin, 2010). Feeding too small a pellet results in inefficient feeding because more energy is used in finding and eating more pellets. Conversely, pellets that are too large will depress feeding and, in the extreme, cause choking. In general it is a good practice to select the largest sized pellet the fish will actively eat.

FEED FORMULATION

The practical application of fish nutrition is to produce feeds that support growth, health, and welfare of the farmed aquatic animals. This objective is achieved by selecting appropriate feed ingredients, deciding how they should be combined to meet the nutritional requirements of farmed aquatic animals, and processing with a combination or mixture of ingredients into a physical form suitable for practical use. Each step in the process of making fish and shrimp feeds requires specific information, judgment, and compromise. Complete information must be available for feed ingredients being considered as components of feeds, including proximate composition, nutrient contents, quality and potential variability among sources or producers, anti-nutrient contents, contaminant level, and the digestibility of nutrients to farmed fish or shrimp. The nutritional requirements of the fish or shrimps at the life stage for which the feed is being prepared must be known. Potential interactions among feed ingredients that might influence the bioavailability of essential nutrients must be considered, as well as the physical characteristics of feed ingredients mixtures that might affect how it can be processed into pellets. Processing feed ingredient mixtures into pellets also requires careful consideration. Physical characteristics of pellets, such as hardness and durability, water stability, and porosity, are determined by the blend of ingredients used in a feed mixture and by the processing techniques used during conditioning of the ingredients mixtures and pelleting. Further, processing techniques increase the availability of some nutrients in feeds but decrease the availability of others.

Ingredients for feed formulation could be chosen from a commonly used list of ingredients (Table 5) when preparing a supplemental feed, so that a feed mixture having the desired crude protein content is obtained. Inquire from poultry and livestock dealers and farmers what ingredients are locally available. There are several methods for formulation of fish feeds according to the fish requirements and onsite needs of the farmers. The most commonly used methods for feed formulations are been provided below.

METHOD 1: ***Trial and error method***

1) Choose a combination of ingredients from Table 5 that will provide a feed containing 25 to 30% crude protein.

Table 5: Composition of commonly used ingredients in fish feeds

Ingredient	**Amount of Ingredients (%)**	**% Crude Protein**	**Crude Protein in Feed (kg)**
Rice bran	47	10	4.7
Corn Gluten (CG 30)	2	30	0.6
Rapeseed meal	7	37	2.6
Soybean Meal	30	46	13.8
Fish Meal	8	65	5.2
Sunflower	4	25	1.0
Total	100		27.9

METHOD 2: ***Pearson's Square***

The Pearson square method of balancing rations is a simple procedure that has been used for many years. It is designed for simple rations with limited options in terms of nutrients and feedstuffs. The following are a few considerations for a Pearson Square;

1. This method allows calculation of one nutrient need at one time using two feeds.
2. The major limitation of using this method is that the level of nutrient being computed must be between the nutrient levels of the two feed ingredients being used. For example, the 30%

crude protein (CP) requirement has to be intermediate between soybean meal (that has 46% CP) and corn (that has 10% CP). If barley is used that (that has 12% CP) and corn (that has 10% CP), this calculation method will not work because the 30% is outside the range of values of two ingredients.

3. Disregard any negative numbers that are generated on the right side of the square. Be concentrate only with the numerical difference between the nutrient requirement and the ingredient nutrient values.
4. This method cannot handle inequalities and both are independent of price.

Diet formulation with the Pearson square method is very simple. This method may be used for two or more feed ingredients and is preferable to the trial and error method. Examples of feed formulations with two and more ingredients are shown.

Example 1. ***Two Ingredients***

Find the proportions of rice bran and soybean meal required to make a feed containing 30 % crude protein.

1. Draw a square (Fig.11 see below).
2. Place the desired protein level at the center of the square. In this case is 30 %.
3. Place the two ingredients on the two left corners of the square along with the protein content of each.
4. Calculate the difference in crude protein content of the two ingredients (46 and 11) and record this number (35) near the lower left corner of the square.
5. Subtract the desired protein level (30 %) of the feed from the protein content of each ingredient and place the answer in the corner diagonally opposite from each ingredient. Ignore positive or negative signs. The difference between percentages of protein

in rice bran and in the feed (19) represents the amount of rice bran needed. The difference between soybean meal and the feed (16) represents the amount of soybean meal needed.

6. Add the differences obtained at the right corners of the square (19 and 16) and record their sum (35) near the bottom right corner. The sum in the right corner should equal the difference in crude protein content recorded near the lower left corner of the square.

7. Divide the sum obtained in step 6, which was 35, into each difference obtained in step 6, which were 19 and 16. Then multiply each by 100 to obtain the percentage of each ingredient needed for the feed.

Thus, 45.7 kg of soybean meal and 54.3 kg of rice bran are combined to make 100 kg of fish feed containing 30% crude protein. The feed can also be described as being composed of 45.7 % soybean meal and 54.3 % rice bran.

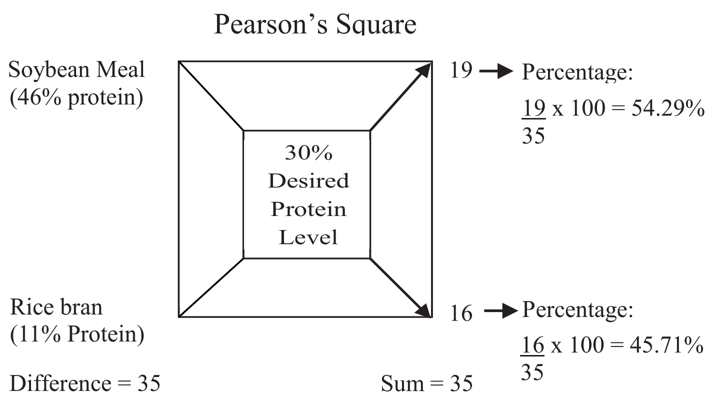


Figure 11. Pearson's Square calculations (using two ingredients).

Example 2. ***Three or More Ingredients***

In this example, find the proportions of soybean meal cake, fish meal, ground corn and cassava flour needed to make a fish feed with a 30% crude protein content.

1. Draw a square and place the desired protein level (30%) at the center of the square. (See Figure 13).
2. Group the ingredients into energy sources (crude protein less than 20%) and protein supplements (crude protein greater than 20%).
3. Calculate an average for the crude protein (CP) contents of each group of ingredients.
4. Place the averages obtained above at the left corners of the square.
5. Calculate the difference in crude protein content between the protein supplements and energy sources and record this near the lower left corner of the square. The answer in this case is 46.

Pearson's Square 2

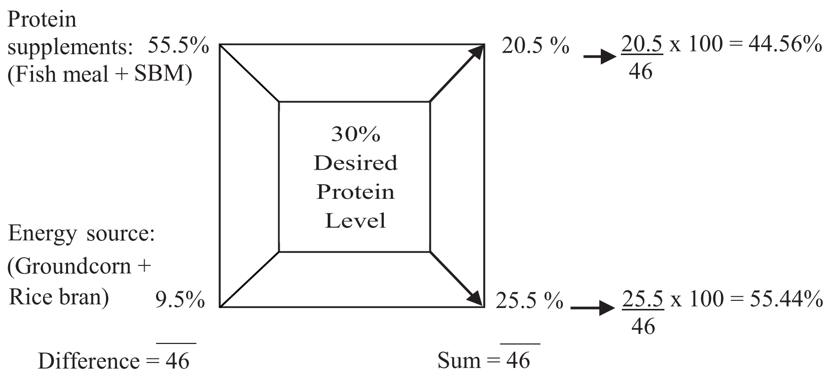


Fig.12. Pearson's Square calculations (using two or more ingredients).

6. Subtract the desired protein level (30 %) of the feed from the combined protein content of the protein supplements and energy sources and place the difference in the opposite diagonal corners. Ignore positive or negative signs. Results are 20.5 and 25.5, respectively for the protein supplements and energy sources.

	Ingredients	CP (%)
Protein Supplement:	Fish Meal	65.00%
	SBM	46.00%
	Total	111.00%
	Average:	55.50%

	Ingredients	CP (%)
Energy Source:	Ground Corn	9.00%
	Rice Bran	10.00%
	Total	19.00%
	Average:	9.50%

7. Add these differences and record the sum near the lower right corner of the square. In this case, the answer is 46 on the left side and 46 on the right side.

8. Divide the left side sum (46) into each difference obtained in step 6 (20.5 and 25.5) and multiply by 100 to calculate the percentage of protein supplement and energy source needed for the feed. The respective answers are 44.56% and 55.44%.

9. One half of the protein supplement (20.5%) is provided by fish meal and one half is provided by soybean meal. One half of the energy source (25.5%) is provided by ground corn and one half is provided by rice bran.

Thus, to make 100 kg of fish feed containing 30% crude protein from a combination of ingredients including fish meal, soybean meal, and ground corn and rice bran the following proportions would be mixed:

Fish meal	27.72 kg
Soybean Meal	27.72 kg
Ground corn	22.28 kg
Rice bran	22.28 kg

METHOD 3: *Spreadsheet Formulations*

Spreadsheet base feed formulation program can provide a fast and accurate way to solve both linear and non-linear feed formulation problems. These feed formulation spreadsheets are relatively easy to build and manage and can be altered manually (e.g. change in feedstuff inventory or the composition and prices of feedstuffs). The expertise is required to build and manage a spreadsheet based on computerized feed formulation program are not high, but include basic expertise in using spreadsheet program, feed composition, requirement tables and their prices. Spreadsheet based software commonly used are Microsoft Excel or Quattro Pro. Advantages to use spreadsheet based feed formulation program are:

1. Spreadsheet based feed formulations program usually generate nutrients requirements after entering the animal description, thereby avoiding the need to look up nutrient requirements from a table.
2. These programs provide a list of feed ingredients and their nutrients composition.
3. Computer programs show the balance all nutrients simultaneously.

4. Because the formulas are built into the program, the chances of mathematical mistakes are eliminated.
5. Spreadsheet based programs can be learned in a much shorter period than specialized feed formulation programs.
6. Spreadsheet program are much less expensive than commercial feed formulations software. In contrast, besides being expensive, sophisticated feed formulation software may be difficult to learn.
7. With the use of macros, spreadsheets could be used to create 'turnkey' feed formulation application.

Spreadsheets are easy to customize, so that features can be added by operators with minimal training. For example, equations can be added to the spreadsheet to calculate prices that could be expected from changing an ingredient. Small producers benefits from using spreadsheets to formulate diets, and can tailor the spreadsheets to fit their situation.

Construction and solving of linear feed formulation problems in spreadsheets is not as easy as with programs designed specifically for feed formulation. But the process is much easier than with dedicated programs because of the capability and flexibility of modern spreadsheet program. Microsoft Excel comes with a programming capability of "SOLVER" function which has linear programming functionality that can be used in least-cost ration formulation. The basic principle of a spreadsheet based feed formulation program is to simultaneously solve a series of equations by minimizing or maximizing parameters such as feed cost and /or nutrients levels.

Least Cost Feed Formulations

Least cost feed formulations for fish on the other hand are a recent innovation. There are still many gaps in our knowledge regarding fish nutrition and digestibility of common feedstuffs by cultured species of fish. Among the few species where such knowledge is more complete, LP techniques for diet formulation have been attempted.

As least cost formulation is a mathematical solution based on the linear programing, it requires the knowledge of animal nutritionists to consider the nutrient requirement of the targeted animal and its capability to digest and assimilate nutrients from available feeds. Linear programing performance is based on information entered by the formulator, and so the formulation is only as good as the nutrient and ingredient parameters entered. However, the program is incapable of correcting errors due to incorrect input data. A few advantages of using linear programming for feed formulation included.

1. This formulation software solves simple to very complex problems such as applications for formula costing, inventory control, control of usage of ingredients in limited availability.
2. Specialized feed formulation program also have built-in nutrient requirements and feed composition tables. Most programs also have the option to allow user to build personalize feed stuff libraries.
3. Linear programming based feed formulation software has many options, which give the formulator choices during formulation, such as calculation of shadow (or opportunity) prices.

Least-cost feed formulation combines many feed ingredients to provide the target animal with a balance nutritional feed at the least possible cost. A fundamental rule of least cost diet formulation software is that adding extra constraints can only maintain or increase the minimum diet cost.

A number of software programs in the market are using linear programming of feed formulation, examples;

Brill Formulation: http://www.feedsys.com/
Feedlive: http://www.liveinformatics.com
Feed Mania: http://sos.saltbush.une.edu.au
MIXIT: http://www.softsearch.com/product1.asp?Product_ID=310008
Winpas: http://www.winpas.com
Winfeed : http://www.winfeed.com

The goals of feed formulation and manufacture are to enhance fish production and ensure its health. Prior to feed formulation, we have to ascertain whether it is a *research diet* or *production diet*. Research di*et al*ways composes pure nutrients while practical diet is formulated from practical ingredients. In the presence of natural food, there is no need to provide complete feed, however, supplementary feed with comparatively low nutrition value can be provided to enhance fish growth while in intensive fish culture complete diet is pre-requisite for sustainable fish production. Following factors must be considered before starting feed formulation;

1) Nutrient requirements of fish
2) Nutrient content of feed ingredients and level of anti-nutritional factors
3) Cost of feed ingredients
4) Availability of nutrients to fish from various feed materials
5) Minimum maximum restrictions on levels of various ingredients

Factors 2 and 3 are readily available for most commercial feedstuffs while factor 1, nutrient requirements are known for several species (National Research Council, 1992, 2011). Management, environmental factors and fish size can have an effect on nutrient requirements. If a nutrient requirement for particular species is unknown, data from related species can be used. Generally most variation is expected between warm and cold water, fresh and salt water, finfish and crustaceans. Refinement in nutrient requirements and allowances will make feed cost effective. Over fortification of labile nutrients is required. For example, up to 50% of vitamin C is lost during processing and its shelf life is 3 months. Particle size should be large enough to minimize nutrient losses. Feed texture, dry or moist, most fishes accept dry but eels and young salmons prefer soft diets. Nature of feed, whether floating, sinking, slow sinking or sinking is required.

The 4th factor considered essential for feed formulation is availability of nutrients from feed offered. Digestibility of energy from CHO is much less for cold-water fish than warm-water fish. Digestibility of phosphorus is less for fish than for livestock. Lysine in cottonseed meal is only 75% as digestible as the lysine in soybean meal. During formulation we fix the upper and lower limits for certain nutrients due to their cost availability to fish and toxicity if provided over than requirements. Same is the case with feed ingredients. These restrictions are imposed due to several reasons; cost, nutrient availability and level of toxins are a few to mention.

Table: 6. Example Nutrient restrictions for the least-cost feed formulation for grow-out of Nile Tilapia

Item	Restriction	Quantity [a]
Crude Protein (%)	Minimum	32.0
Crude Fiber (%)	Maximum	7.0
Lipid (%)	Maximum	6.0
Linolenic series (n-6) fatty acid	Minimum	1.0
Available phosphorus (%)	Maximum	0.5
Digestible energy ($kcal.g^{-1}$)	Minimum	2.8
Digestible energy ($kcal.g^{-1}$)	Maximum	3.1
Available lysine[b]	Minimum	1.6
Available methionine[b]	Minimum	0.51
Available methionine + cyctine[b]	Minimum	1.02
Grain or grain by-products	Maximum	25.0

Cotton meal (%)	Maximum	15.0
Whole fish meal or other high quality animal protein source	Minimum	6.0
Soybean meal	None	Unlimited
Vitamin premix[c]	Fixed	
Trace mineral premix[c]	Fixed	

[a] Quantity expressed on an as-fed basis (about 10% moisture). [b] Quantities vary depending on dietary level of protein. [c] Must meet recommendation.

Sources: Chhorn Lim, Carl D. Webster, Tilapia: Biology, Culture and Nutrition, 2006. pp 530.

By following the described nutrient restrictions, a sample of least cost formulation for tilapia has been provided below for guidance about usage of dehulled soybean meal combination of locally available feed ingredients in Pakistan (Dr. Zafar Khan and R.S.N. Janjua dated December 11, 2014.)

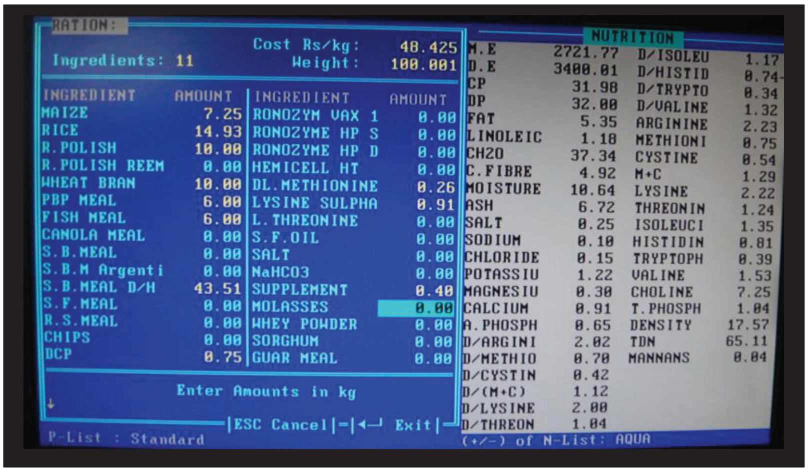


Figure 13. Picture taken by R.S.N. Janjua, ASA/WISHH/PK, December 11, 2014

Table 7a and 7b. Recommended Formula for Tilapia grow out period feed is prepared at digestible energy (DE) 3,400 Kcal/Kg and digestible protein (DP) at 32% as per nutrient values specified by NRC-2011

	Ingredient	Inclusion %	Specified Min%	Limits Max%	Prices Rs./ kg [a]	Batch Wt. kg	Cost of Batch Wt (Rs.)
1	Maize	7.249	-	-	25.00	7.25	181.23
2	Rice	14.928			26.77	14.93	399.62
3	Rice Polish	10.000	-	10.00	19.24	10.00	192.40
4	Wheat Bran	10.000	-	10.00	22.64	10.00	226.40
5	Poultry by-products Meal	6.000	-	6.00	54.42	6.00	326.52
6	Fish Meal	6.000	6.00		77.50	6.00	465.00
7	Canola Meal	-	-	-		-	-
8	SBM (Dehulled)	43.509	-	50.00	65.00	43.51	2,828.09
9	Di-Calcium Phosphate	0.753	-	-	45	0.75	33.89
10	DL Methionine	0.257	-	-	387.00	0.26	99.46
11	Lysine Sulfate	0.905	-	-	99.00	0.91	89.60
12	Supplement	0.400	0.40	0.40	1.00	0.40	0.40
	Total	100.00				100.00	4,842.59
	Cost of Feed per kg						48.43

[a] The price of raw materials as of dated December 11, 2014

Table 7b.

S. No.	Nutrient	Amounts	Units	Specified Min%
1	Digestible energy	3,400.00	K.cal/kg	3,400.00
2	Digestible protein	32.000	%	32.00
3	Calcium	0.905	%	0.65
4	Phosphorus	0.650	%	0.65
5	Arginine	2.022	%	1.00
6	Methionine	0.700	%	0.70
7	Cystine	0.417	%	0.30
8	(Methonine + Cystine)	1.117	%	1.00
9	Lysine	2.000	%	2.00
10	Threonine	1.044	%	1.00
11	Isoleucine	1.17	%	1.05
12	Histidine	0.74	%	0.50

Table 8a and 8b. Optimum Formula for Carp is developed with reference to specified minimum nutrient in NRC, 2011.

S. No	Ingredient	Inclu-sion %	Speci-ied Min%	Limits Max%	Prices Rs. / kg	Batch Wt. kg	Cost of Batch Wt (Rs.)
1	Maize	16.453	-	-	25.00	16.45	411.325
2	Rice Polish	10.000	-	10	19.24	10.00	192.40
3	Wheat Bran	13.000	-	13	22.64	13.00	294.32
4	Poultry Byproducts Meal	5.000	-	6	54.42	5.00	272.10
5	Fish Meal	4.000	4		77.50	4.00	31.00
6	Canola Meal	10.000	-	10	43.95	10.00	439.50
7	Soybean Meal (Dehulled)	37.527	-	-	65.00	37.53	2439.255
8	Dicalcium phosphate	1.328	-	-	45	1.33	59.76
9	Methionine	0.271	-	-	387.00	0.27	104.877
10	Lysine Sulfate	1.039	-	-	99.00	1.04	102.861
11	Threonine	0.982	-	-	200.00	0.98	196.4
12	Supplement	0.400	0.4	0.4	1.00	0.40	0.4
13	Total	100.000				100.00	4,823.20
14	Cost of Feed per kg						48.23

Note: Prices of the feed are variable subject to the price and availability of ingredients.

Table 8b.

S. No.	Nutrient	Amounts	Specified Min Requirements	Units
1	Digestible energy	3256.778	3,250.00	K.cal/kg
2	Digestible protein	32.00	32.00	%
3	Calcium	0.70	0.70	%
4	Phosphorus	0.70	0.70	%
5	Arginine	1.13	1.00	%
6	Methionine	2.00	1.50	%
7	Cystine	0.40	0.20	%
8	(Methonine+Cystine)	2.40	1.70	%
9	Lysine	1.32	1.00	%
10	Threonine	2.21	1.55	%
11	Isoleucine	2.17	1.00	%
12	Histidine	0.80	0.75	%

Table 9a and 9b. Optimum Formula for Trout is prepared at digestible energy (DE) 4,200 Kcal/Kg and digestible protein (DP) at 38% as per nutrient values specified by NRC-2011

S. #	Ingredient	Inclu-sion %	Speci-fied Min%	Limits Max%	Prices Rs./ Kg	Batch Wt.Kg	Cost of Batch Wt (Rs.)
1	Rice	14.185			26.77	14.19	379.73
2	Poultry Byproducts Meal	6 .000	-	6.00	54.42	6.00	326.52
3	Fish Meal	3.899	8		77.50	13.90	1,077.17
4	Soybean Meal (Dehulled)	8.576	-		65.00	48.58	3,157.44
5	Corn Gluten (CG 60%)	5.000	-	5.00	60.00	5.00	300.00
6	Sunflower. Oil	10.178			144.00	10.18	1,465.63
7	DL. Methionine	0.938	-	-	387.0 0	0.94	363.01
8	Lysine Sulphate	0.284	-	-	99.00	0.28	28.12
9	Supplement	0.400	0.4	0.40	1.00	0.40	0.40
	Total	99.46				99.46	7,098.02
	Cost of Feed per kg						70.98

Note: Prices of the feed are variable subject to the price and availability of ingredients.

Importance of energy to protein ratio in feed formulations

Dietary nutrients are essential for the construction of living tissues. They also are a source of stored energy for fish digestion, absorption, growth, reproduction and the other life processes. The nutritional value of a dietary ingredient is in part dependent on its ability to supply energy. Energy is not a nutrient in itself, but is required to drive chemical reactions for tissue maintenance, growth and activity. Too much energy in the feed reduces feed intake and

Table 9b.

S.#	Nutrient	Amounts	Specified Min Requirements	Units
1	Digestible energy	4,200.00	3,400.00	K.cal/kg
2	Digestible protein	32.000	32.00	%
3	Calcium	0.700	0.70	%
4	Phosphorus	2.309	2.00	%
5	Arginine	2.000	2.00	%
6	Methionine	2.000	2.00	%
7	Cystine	0.305	0.30	%
8	(Methonine+Cystine)	2.305	2.30	%
9	Lysine	2.173	1.00	%
10	Threonine	2.235	1.55	%
11	Isoleucine	2.100	2.00	%
12	Histidine	0.868	0.80	%

result into fatty fish which reduces dress out yield and shortens shelf-life of frozen products. Energy/protein ratios should be balanced for best results. Ratio of 8.5 – 9.5 Kcal/g protein is considered adequate for majority of culturable fish species (Dabrowski and Guderly, 2005).

Physiological fuel values are used to calculate and balance available energy values in prepared diets. They typically average 4, 4, and 9 Kcal/g for protein, carbohydrate and lipid, respectively (Lee and Putnam, 1973). To formulate and prepare an optimum diet, the ratio of protein to energy must be determined separately for each fish species. Excess energy relative to protein content in the diet may result in high lipid deposition. Because fish feed to meet their energy requirements, diets with excessive energy levels may result in decreased feed intake and reduced weight gain. Similarly, a diet with inadequate energy content can result in reduced weight gain because the fish cannot eat enough feed to satisfy their energy requirements for growth. Properly formulated prepared feeds have a well-balanced energy to protein ratio. Because feed formulation is the process of combining ingredients to meet the nutritional needs in terms of protein and energy of farmed animals, birds or fish to produce a mixture that can be pelleted, shipped, and stored; relatively water soluble; supports growth, health, and wellness; and is economical to use.

Dietary requirements of commonly cultured fish species

Of the carp species, most nutritional studies have been conducted on the omnivorous common carp. The known dietary requirements of Tilapia, selected cyprinids and catfishes have been given below;

Table 10. Nutritional guidelines for common carp

Fish Species	Digestible Energy	Crude Protein	Fats	Carbohydrate	Amino Acids	Available Phosphorus	Fiber	Essential fatty acids
Common Carp	Kcal / kg	(%)	(%)	(%)	Lysine + Methionine (%) of dietary protein	(%)	(%)	(%)
Fry to 0.5 g	2700 - 3100	25 - 38	18	-	5.7 + 3.1	0.6-0.7	8.0	At least 1% each of

Source: ADCP 1980

Table 11. Indian and Chines Major Carps

Fish Species	Digestible energy	Crude Protein	Fats	Carbohy-drates	Amino Acids	Available Phosph-orus	Fiber	Essential fatty cids
Indian and Chinese Carps	Kcal / kg	(%)	(%)	(%)	Lysine + Methionine (%) of dietary protein	(%)	(%)	9%)
Fry to fingerling s (0.5 -5.0g)	3100	30	8	25	6.7 + 4.0	0.6-1.0	-	-
Juvenile (5 – 100g)	2800	25	5		6.4 + 3.6	05.-1.0		
Grower (100 and onward to market size	2800	25	5		6.0 + 3.3	0.6-1.0		

Source: Aquaculture Development Coordination Program (ADCP); Fish Feeds and Feeding in Developing Countries. ADCP/REP/83/18UNDP, FAO, Rome (1983) and New, 1987

Table 12. Tilapia nutritional requirements.

Fish Species	Digestible energy	Crude Protein	Fats	Carbo-hydrates	Amino Acids	Available Phosph-orus	Fiber	Essential fatty acids
Tilapia	Kcal / kg	(%)	(%)	(%)	Lysine + Methionine (%) of dietary protein	(%)	(%)	9%)
Fry to 0.5g	2500 - 3400	50	10	25	4.45^{a} + 1.7	0.6-0.7	8.0	**less than 1% each of n-3 and n-6**
0.5 – 35gm		36	8					
35 g to market size		32	6				8.0-10	

[a]In presence of cystine at 0.54 percent of dietary protein
Source: Jauncey, 1982.

Table 13. Catfishes

Fish Species	Digestible energy	Crude Protein	Fats	Carbo-hydrates	Amino Acids	Available Phosphorus	Fiber	Essential fatty acids
Channel catfish	Kcal/ kg	(%)	(%)	(%)	Lysine + Methionine (%) of dietary protein	(%)	(%)	(%)
Fry to fingerling (0.5 - 5.0g)	2700-3100	35-40	8-10	Higher than 20% can be tolerated	5.1 + 2.3	0.5	-	- At least 7.5% fish to meet n-3 and n-6 FA
Juvenile (5 – 100g)		35						
Grower (100 and onward to market size		25						

Source: Aquaculture Development Coordination Program (ADCP); Fish Feeds and Feeding in Developing Countries. ADCP/REP/83/18UNDP, FAO, Rome (1983) and New, 1987.

Table 14. Rainbow Trout

Fish Species	Digestibl e energy	Crude Protein	Fats	Carbo-hydrates	Amino Acids	Available Phosphorus	Fiber	Essential fatty acids
Rainbow Trout	Kcal/ kg	(%)	(%)	(%)	Lysine + Methionine (%) of dietary protein	(%)	(%)	(%)
Fry to fingerling (0.5 -5.0g)	2800-4200	50	15	Higher than 20% can be tolerated	5.1 + 2.3	>0.8	-	- At least 1% each of n-3 and n-6
Juvenile (5 – 50g)		40	12					
Grower (50 and onward to market size		32-35	12					

Source: Sedgewick 1982

COMMONLY USED FEED INGREDIENTS IN FISH FEEDS AND THEIR MAIN CHARACTERISTICS

Detail of various ingredients with their main characteristics has been provided in the following paragraphs. However for detailed chemical composition the reader may consult NRC 2011.

Fish Meal

It is the highest quality protein source and is rich in energy and minerals. It is highly palatable and highly digestible. Fish meal from whole fish contains 60-80% protein and is 80 to 95% digestible. It is high in lysine and methionine. Marine fish meal contains 1-2.5% n-3 fatty acids. Fish meal prepared from fish parts is low in protein. It is high in ash and can make unavailability of other nutrients, hence should be used prudently. Other than meeting nutrient requirements it helps to increase growth. Because of its high cost it is used sparingly however, it is mandatory for eels, pen reared salmon and most of other carnivorous fish varieties. Raw fish meal contains thiaminase which destroy thiamin.

Meat and bone meal

It is a product of partially fat-extracted, dried slaughterhouse wastes. It usually contains 50-55% crude protein with significant protein from bone and other non-muscle tissue. Quality of protein is less than whole fish meal and varies with composition of waste material. It is high in ash and hence should not be used in fish feeds in unlimited quantity. It is good source of energy, phosphorus and trace minerals. Flash or spray dried blood meal is highly digestible and rich in protein (80-86%). It is deficient in methionine but rich in lysine. It is not good mineral supplement as fish meal or meat meal because of absence of bones. It is unpalatable to some species.

Poultry by-product meal

Without feathers it is a good protein source for fish feeds. It is usually recycled in poultry feeds. Feather meal is a concentrated protein source (80% CP) but quality is poor. Its digestibility is low unless it is thoroughly hydrolyzed during processing.

Crustacean meal

It is useful feed ingredient if heads are included. Exoskeleton is primarily chitin and has limited nutritional value. Visceral organs in head section are most valuable nutritionally. Percentage protein present in it needs to be corrected for nitrogen because chitin contains 10-15% of the total nitrogen. In addition to protein it is source of n-3 fatty acids, cholesterol (essential for crustaceans) and astaxanthin (for red pigment in salmonids). It is highly palatable and may serve as attractant in fish and crustacean feeds. Krill (*Euphausia pacifica*) is a small marine crustacean harvested from the sea and is made into meal for fish, mammal and poultry. It contains less than 40% CP and is high in chitin and oil and is rich source of n-3 fatty acids. It is valuable source of astaxanthin and is used as a pigment enhancer in skin and flesh.

Soybean Meal

Soybean meal (SBM) has one of the best amino acid profiles of all protein-rich plant feedstuffs for meeting essential amino acid requirements of fish. It is not deficient in any essential amino acid but for carnivores it is. It is deficient in threonine for eel. It is unpalatable to some fish such as coho salmon. For rainbow trout it provides satisfactory growth but when level of fish meal was reduced below 18% of diet, palatability was reduced. Solvent extracted SBM is used up to 60-70% of the formula in channel catfish. Dehulled SBM contains 25% less metabolizable energy 86% less available phosphorus and 90% less n-3 fatty acids than fish meal however, these values vary from species to species e.g. apparent digestibility energy in rainbow trout for fish meal is 99%, for silver perch 89-98% and for tilapia it is 89% while for SBM digestible energy values for the same species are 87, 78 and 84% respectively (NCR, 2011). It contains several anti-nutritional factors e.g. trypsin inhibitor and phytic acid. During oil extraction SBM is heated at 105 $^{\circ}$C for 10-20 min, and if properly cooked the entire anti-nutritional factors are inactivated to increase the nutritional value.

Full Fat Soybean Meal

Full fat soybean is approximately 18% fat and 37% protein. Ground full fat soybean meal can be successfully used in the formulated feeds of ruminants, but it poorly digested in mono-gastric animals like fish. However extruded full fat soybean meal has higher digestibility values both for protein and energy and can significantly replace animal protein source in fish feeds with reasonable growth. Its higher level of protein and essential amino acids make it an ideal ingredient to make feed for trout, tilapia and other fish.

Cotton Seed Meal

It is an important source of protein in many parts of the world. Initially it was a major source of protein but with the inclusion of other ingredients its use is eliminated due to low level of lysine, however, addition of synthetic amino acids can improve its value. Moreover, presence of free gossypol is moderately toxic to mono-gastric animals. Gossypol at 0.03% or greater suppresses growth in rainbow trout, while levels higher than 0.2% are needed to cause toxicity in channel catfish. Levels of free gossypol depends on processing, e.g. direct solvent meal contains 0.2-0.4%, while screw pressed contains 0.02% and prepress solvent extracted meal contains 0.05 % gossypol. Addition of 0.85:1 part of $FeSO_4$ to each part of gossypol has blocked toxic effects of gossypol in swine and poultry.

Other Oil Seed Meals

Peanut and sunflower meals are also used in fish feed. Compared to SBM they are deficient in methionine and lysine. Although peanut meal contains 48% protein; it is highly palatable but has limited applications in fish feed due several issues like rancidity and completion with human being. Sunflower meal is also palatable but relatively fibrous. Canola meal is comparable to SBM in protein quality contents, however, it contains glucosinase enzymes, which hydrolyze glucosinolate to yield anti-thyroid products making it poor protein source.

Grains and their by-products

They are primarily source of carbohydrates in feed. Whole grains contain 62-72% starch which is 60-70% digestible by warm water fishes and less than 40% digestible in salmonids. It's heating increases digestibility up to 10-15%. Starch in grains is valuable binding agent in steam pelleted and extruded fish feeds. Yellow corn by-products are most widely used in fish feeds in USA. It is low in protein and protein is poor in amino acid balance. It contains 20-30 mg/kg of yellow pigment xanthophylls (leutin and zeaxanthin) which impart undesirable yellow color to some areas in white fleshed fish. Approximately up to 11 mg xanthophylls /kg of feed can be used in catfish without imparting any adverse effect on pigmentation of flesh. Corn gluten meal contains 40-60% protein and is a good source of methionine. However, it is concentrated source of (200-350 mg/kg) xanthophylls. Corn gluten meal from distilleries and breweries, is relatively high in protein (26-28%) and lipid (8-10%) but low in lysine.

Rice bran and wheat brans

Rice bran typically includes rice polishing and contains approximately 12% protein, 12% fiber and 12% lipid. It is normally available at reasonable cost. It is good source of nutrients but does not pellet well due to its high lipid and fiber contents. Some people feel that wheat is too valuable for human food to feed to fish, nevertheless a small amount is used as binder. Wheat gluten (protein)-is an excellent binder. Wheat bran and middlings is commonly used in salmonid feeds, because they contain more protein and less starch than whole wheat.

Fibrous Feedstuffs

Fish do not require fiber in feeds and being monogastric cannot digest fibrous feeds well. Addition of fiber to 3-5% feed containing fiber does not have any benefit to fish. In most cases concern is to avoid excessive fiber because high fiber reduces binding quality of feeds, inhibits feed intake and increase fecal waste production.

Fats and Oils

They are used as energy source and provide essential fatty acids. They are used for outer coating of the pellets to reduce abrasiveness (resulting in fewer fines) and minimize dustiness. Fats from livestock and poultry are highly saturated but are effective source of energy for warm- and cold-water fishes. Vegetable oils are expensive because of their usage in human foods but are also good energy source for fish. Marine fish oils are used in trout, salmon and other cultured marine fishes because they are source of essential fatty acids (EFAs). Marine fish oils contain on the average 20-25% long chain (over 20 C) n-3 fatty acids. Animal and vegetable oils do not contain fatty acids beyond 18 C long and except for SBM and linseed oil, most are low in n-3 fatty acids. Channel catfish oil though it is recycled but has the same composition as animals.

Nutrient and Energy Utilization

The fractions of dietary nutrients or energy that are eliminated in the feces represent undigested components that do not contribute to the nutrition of the fish. So it is generally desirable to use feeds that have a high level of digestibility. Coefficients of nutrient and energy digestibility for complete feeds or specific ingredients can be used to assess the relative percentage of ingested nutrients that are retained by the fish. Digestibility coefficients for specific feedstuffs can help producers more precisely formulate feeds to meet the nutrient requirements of the cultured species. This information is now available for many common feedstuffs and established fish species.

Nutrient Deficiency Diseases

In culturing fish in captivity, nothing is more important than sound nutrition and adequate feeding. If there is no utilizable feed intake by the fish, there can be no growth and death eventually results. Under-nourished or malnourished animals cannot maintain health and growth, regardless of the quality of the environment. Therefore, before any attempt at fish culture it would be wise to ask a fundamental question, "What and how should I feed my fish?" Faulty nutrition impairs fish productivity and affects their health; a fact the fish culturist should always keep in mind. Clinical disease often ensues when nutritional needs are not met. The borderline between reduced growth and diminished health and overt disease is difficult to define. Diets may hasten recovery from infection or slow the progress of an idiopathic disease or overcome environmental stress. However, diets may also cause nutrient imbalances, deficiency diseases, nutritional toxicosis, or may introduce infective agents. As a consequence, nutritionally- balanced and quality-controlled diets are of critical concern in fish production.

PROTEINS

Protein provides a major source of energy for fish and subsequently fish require a higher percentage than warm blooded animals e.g. 30 to 36% for warm water fish vs. 16 to 22% for poultry. Protein requirements vary with fish species and developmental stage of fish. The minimum dietary requirement for protein or balanced mixture of amino acids is of primary concern in aquaculture because it can ensure growth and health of fish.

Dietary proteins are the source of essential amino acids and provide nitrogen for the synthesis of non-essential amino acids. Proteins in the body tissues are composed of about 23 amino acids. Out of these, 10 are essential amino acids. Proteins or amino acids are necessary for maintenance, growth, reproduction and replacement of depleted tissues. In addition, certain amino acids are readily converted to glucose to provide an essential energy source for some critical body organs and tissues such as brain and red blood cells. Since carbohydrate is not prevalent in their natural diet, fish are more dependent upon amino acids as precursors to glucose than most other animals. Therefore, a portion of the dietary protein is always used as an energy source in fish. Not all dietary proteins are identical in their nutritional value. To a large extent, the bio-availability of a protein source is a function of its digestibility and amino acid makeup. Some protein feedstuffs which contain a high level of crude protein (% total nitrogen x 6.25) are low in amino-nitrogen and do not contribute toward the requirement of amino acids. As a result, such materials may merely increase ammonia production into the water environment. A deficiency of essential amino acids may lead to poor utilization of dietary protein, and may result in growth retardation, poor live weight gain, and low feed efficiency. In severe cases, amino acid deficiency lowers resistance to diseases and impairs the effectiveness of the immune response mechanism. Deficiencies of specific amino acids may also elicit clinical signs. For example, experiments have shown that tryptophan deficient fish become scoliotic, showing a characteristic curvature of the spine (Kloppel and Post 1975) and a methionine deficiency is one cause of lens cataracts (Postonet *et al*. 1977). The protein component generally represents the largest portion of the total cost of a diet, and hence is always restricted which ultimately ends up in various deficiency signs ultimately affecting the production of fish farming unit. But protein ingredients are not necessarily as expensive as protein is.

This paragraph that follows will provide some information on the relationship of protein and lipids, some safe ingredient inclusion level, dietary protein requirements and some energy to protein ratios which can prevent deficiency signs and streamline the desired production. Under certain market conditions, using protein, rather than lipid, as an energy source may reduce feed cost. Major sources of protein for salmonid diets are marine fish meals (in Canada - herring and whole capelin meals). In most diet formula, other protein ingredients such as soybean meal, corn gluten meal, and dairy or animal by-product meals are employed as protein sources. In feed formula for fry, broodstock, and fingerling fish, 30-50 % of high quality fish meal and 10-15 % of fish oil are recommended in the diet. The cost of feed is a relatively small fraction of the potential economic value of the fish produced. Most salmonids diets should contain 56-75 g of amino-nitrogen per kg of feed; this is equivalent to 35-50 % crude protein. However, amino acids or protein must be supplied in relation to the needed energy content. The recommended ratio of protein to energy in salmonids diets is 3.5-4.0 g digestible amino-nitrogen per MJ digestible energy (15-17 g N or 92-105 g protein per $Mcal^{-1}$). Ratios in excess of these values result in increased ammonia excretion. Furthermore, the dissolved oxygen requirement increases as energy efficiency is decreased.

LIPIDS

Fatty acids are the main constituents of dietary lipids. Fish and mammals appear to be unable to synthesize fatty acids that are unsaturated in the ω-3 or ω-6 positions unless a suitable precursor is supplied in the diet. Thus, the lipid component of the diet must provide an adequate amount of essential fatty acids for growth as well as for required dietary fuel. In contrast to mammals which have a major requirement for ω-6 fatty acids, many cold-water and marine fishes require ω-3 fatty acids. Therefore, sufficient amounts of essential ω fatty acids (ω-3 fatty acids or longer chain members of these series) must be included in the dietary lipids. One percent linolenic acid (18:3 n-3) in the diet is required by rainbow trout to avoid such deficiency signs as loss of pigmentation, fin erosion, cardiac myopathy, fatty infiltration of the liver, and shock syndrome (Castel1 *et al*.1972). Salmonids utilize lipids as a major source of energy and digest complex carbohydrates very poorly. Diets for salmonids, therefore, should contain very high levels of lipids (10-18%) in comparison to diets for other animals. Tilapia do not need such high levels of lipid in the diet, typically 6-7% (Fitzsimmons *et al*. 1997). Because of the high level of use, lipid quality is critical since marine fish oil is very susceptible to oxidation. In all circumstances, rancid oil must be avoided in fish feed. Fish suffering from lipoid liver disease have extreme anemia, a bronzed, rounded heart and a swollen liver with rounded edges. Histologically, the main feature is the extreme lipoid infiltration of hepatocytes and associated loss of cytoplasmic staining and distortion of hepatic muralia (Cowey and Roberts 1978). All salmonids are susceptible to lipoid degeneration of the liver, but it is a particularly significant problem in rainbow trout. Slightly affected fish are usually capable of recovery, but if severe anemia and hepatic ceroidosis have developed, the fish are rarely capable of recovery to an acceptable feed efficiency (Cowey and Roberts, 1978).

VITAMINS

Vitamins are micro-nutrients required for normal growth, reproduction, health and maintenance of fish metabolism. The requirements of fish depend upon the intake of other nutrients, size of the fish, and environmental stresses. Four fat-soluble and eleven water-soluble vitamins are known to be required by the fish and the roles and functions of individual vitamins have been described (NRC 1981, 1993 and 2011). Recommended dietary levels and deficiency signs are summarized in Table 15. In the early days of fish culture, the most common nutritional deficiencies were those associated with vitamins. However, most of today's practical diets contain sufficient quantities of vitamins. In spite of the addition of excess amounts of vitamins to most fish diets, vitamin deficiency disorders still occur in fish culture. The primary reasons are related to improper manufacturing, handling, or storage of fish feed. Vitamins are very susceptible to destruction by oxidation in the presence of excessive moisture, heat, and trace minerals, particularly if rancid fat is present. Many of the vitamin deficiency signs are non-specific and it is difficult and costly to analyze for most of the vitamins. Therefore, the diagnosis of vitamin deficiencies is usually accomplished by a process of eliminating other causes and reviewing information on the diet formula used, the level of supplementation of vitamins and minerals, and the manufacturing and storage conditions (Table 15).

Table 15: Nutritional Deficiency Signs in Finfish

Signs	Possible Nutrient Deficiencies
Anemia	Folic acid, inositol, niacin, pyridoxine, riboflavin, rancid fat, vitamins B_{12}, C, E&K
Anorexia (poor appetite)	Biotin, folic acid, inositol, niacin, pantothenic acid, pyridoxine, riboflavin, thiamine, vitamins A, B_{12} and C
Ascites	Vitamins A, C and E
Ataxia	Pyridoxine, pantothenic acid, riboflavin

Atrophy, gills	Pantothenic acid
Atrophy, muscle	Biotin, thiamine
Calcinosis, renal	Magnesium
Cartilage abnormality	Vitamin C, tryptophan.
Cataract	Methionine, riboflavin, thiamine, zinc
Ceroid liver	Rancid fat, vitamin E
Cloudy lens	Methionine, riboflavin, zinc
Clubbed gills	Pantothenic acid
Clotting blood slow	Vitamin K
Coloration, dark skin	Biotin, folic acid, pyridoxine, riboflavin
Convulsions	Biotin, pyridoxine, thiamine
Decoloration, skin	Fatty acids, thiamine
Deformation bone	Phosphorus
Deformation lens	Vitamin A
Degeneration gills	Biotin
Dermatitis	Pantothenic Acid
Diathesis exudative	Selenium
Disease resistance low	Protein, vitamin C
Distended stomach	Inositol
Dystrophy, muscular	Selenium, vitamin E....
Edema Niacin	Pyridoxine, thiamine, vitamins A and E
Epicarditis	Vitamin E
Erosion fin	Pyridoxine, thiamine
Equilibrium loss	Fatty acids, riboflavin, vitamin A, Zinc
Exophthalmos	Pyridoxine, vitamin A, C and E
Exudative gills	Pantothenic acid
Fatty liver	Biotin, choline, fatty acids, inositol, vitamin E
Feed efficiency, poor	Biotin, calcium, choline, energy, fat, folic, acid, inositol, niacin, protein, riboflavin.......
Fragility, erythrocytes.	Biotin, vitamin E
Fragility fin	Folic acid
Fragmentation erythrocytes	Biotin, vitamins B_{12} and E
Gasping rapid	Pyridoxine
Goiter	Iodine

Growth poor	Biotin, calcium, choline, energy, fat, folic acid, inositol, niacin, pantothenic acid, protein, pyridoxine, riboflavin, thiamine, vitamins A, B_{12},C, D and E
Hematocrit, reduced	Iron, vitamins C and E
Hemoglobin, low	Iron, vitamins B_{12} and C
Hemorrhage, eye	Riboflavin, vitamin A
Hemorrhage, gill	Vitamin C
Hemorrhage, kidney	Choline, vitamins A and C
Hemorrhage liver	Vitamin C
Hemorrhage skin	Niacin, pantothenic acid, riboflavin,
Irritability	Vitamins A and C
Lesion, colon	Fatty acids, pyridoxine, thiamine
Lesion, eye	Biotin, niacin
Lesion skin	Methionine. riboflavin, vitamins A and C, zinc
Lethargy	Biotin, inositol, niacin, pantothenic acid
Lipoid liver	Folic acid, niacin, pantothenic acid, thiamine,
Lordosis	vitamin C
Myopathy, cardiac	Essential fatty acids
Necrosis Liver	Pantothenic acid
Nerve disorder	Pyridoxine, thiamine
Pale liver (glycogen)	Highly digestible carbohydrate, biotin
Photophobia	Niacin, riboflavin
Pinhead	Starvation
Pigmentation iris	Riboflavin
Prostration	Pantothenic acid, vitamin C
Rigor mortis, rapid	Pyridoxine
Scoliosis	Phosphorus, tryptophan, vitamins C and D
Shock syndrome	Essential fatty acids
Slime, blue	Biotin, Pyridoxine
Spasm, muscle	Niacin
Swimming, erratic	Pyridoxine, pantothenic acid
Tetany, white muscle	Niacin, vitamin D
Vascularization, cornea	Riboflavin

Nutritional disorders caused by vitamin deficiencies can impair the utilization of other nutrients, weaken the health of the fish, and lead to disease. It is well known that pantothenic acid deficiency results in nutritional or clubbed gill disease. However, this condition may not be as specific as reported because feeding a diet containing 10 mg pantothenic acid/kg feed (National Research Council 2011) recommends 40 mg/kg) for 5 months did not produce either the described deficiency signs or growth depression in laboratory studies. Vitamin C (Ascorbic acid) is the most unstable vitamin required in fish diets (Hilton *et al*. 1977).

MINERALS

In fish, minerals perform important roles in osmoregulation, intermediary metabolism, and in formation of the skeleton and scales (La11 1981). Mineral requirements of fish are difficult to study because many minerals are required in only trace amounts and others are absorbed from water in significant quantities through the gills as well as from the diet. It is also very difficult to obtain mineral free feed ingredients for experimental diets. Most practical diets for salmonids provide the major mineral requirements through fish meal which is also a major source of protein. However, diets which rely heavily on plant protein sources must be supplemented with carefully balanced mineral premixes. The minerals required in finfish diets include calcium, zinc, manganese, cobalt, selenium, iodine and fluorine. The functions of some of these are available in detail in NRC (1993 & 2011).

TOXINS, ANTIMETABOLITES AND ANTINUTRITIONAL FACTORS

Toxins which may be present in fish feeds include mycotoxins, residues of polychlorinated biphenyls, pesticides, herbicides, other agricultural and industrial chemicals and inherent plant by products. Mycotoxins are produced by many molds on plant products such as oilseed by products (soybean, cotton seed, and peanut meals) and grain byproducts. In particular, aflatoxin B_1 (at less than 1 ppb) in the diet will produce liver cancer in rainbow trout in one year, and at 8-20 ppb will induce grossly visible hepatomas in 4-6 months (Sinnhuber *et al*. 1977). Other toxins and anti-metabolites in plant materials are protease inhibitors, hemagglutinins, goitrogens, cyanogens, saponins, and gossypol (Table 10) (NRC 1993). However, these compounds can be destroyed or inactivated by proper processing (e.g. heating or chemical treatment). Microbial toxins which are produced by microorganisms associated with feed contamination or spoilage cause bacterial toxicoses causes of feeding raw fish. However, when fed pasteurized salmon viscera, effectively eliminated the primary source of infection (Hublou *et al*. 1959).

Table 16. Important anti-nutrient present in some commonly used alternative fish feeds ingredients.

Plant-derived nutrient source	Anti-nutrients
Soybean meal	Protease inhibitors, lectins, phytic acid, saponins, phytoestrogens, anti-vitamins, allergens
Rapeseed meal	Protease inhibitors, glucosinolates, phytic acid tannins.
Lupin seed meal	Protease inhibitors, saponins, phytoestrogens, alkaloids
Pea seed meal	Protease inhibitors, lectins, tannins, cyanogens, phytic acid, saponins, antivitamins
Sunflower oil cake	Protease inhibitors, saponins, arginase inhibitor
Alfalfa leaf meal	Protease inhibitors, saponins, phytoestrogens, antivitamins
Mustard oil cake	Glucosinolates, tannins
Sesame meal	Phytic acid, protease inhibitors
Cottonseed meal	Phytic acid, phytoestrogens, gossypol, antivitamins, cycolopropenoic acid
Leucaena leaf meal	Mimosine

Source: **AQF 621 2011,** Fish Nutrition and Feed Technology, Bunda College of Agriculture, p.37

FEED ADDITIVES

Binding agents

Steam pelleted fish feeds require addition of special binding agents while extruded do not. Binding agents are especially valuable if not essential in crustacean feeds which must remain in water stable for several hours. Organic hydrocolloids, such as carboxymethyl-cellulose, guar gum, agar and alginic acid have been successfully used in laboratory feeds but are expensive for commercial feeds. Commercial binding agents used in livestock and poultry feeds are hemicelluloses, molasses and lignin sulfonates. They are inexpensive and good for food to be consumed within 30 minutes; however, their effectiveness to keep feed intact for several hours is inconsistent. Inorganic binding agents such as sodium and calcium bentonite or aluminum silicate have been found less effective than organic binders. Cooked (gelatinized) starches, used at ≥10% of the diet are good binders and also serve as source of energy. They are highly hygroscopic and will absorb water which causes the pellet to swell and disintegrate after a time in water. Polymethylcarbamide binder (Basfin) has been used successfully in crustacean feeds. At levels of 0.5 to 0.8% with good processing technology; it allowed pellets to remain intact in water for ≥6 hours. This compound is non-hygroscopic and absorb little water, it comfortably cross links with carbohydrate and protein. A level above 0.5% affects palatability and currently is no more in use.

Carotenoid Supplements

Carotenoid supplements can be chemically synthesized products and natural materials like plant extracts, herring gull eggs, salmon eggs, paprika, zooplankton, krill products and processing waste from shrimp crab, and crayfish. Some fishes have the capacity to convert certain xanthophyll pigments to carotenoids. Goldfish and common carp can convert yellow xanthophyll (zeaxanthin) to red carotenoid (astaxanthin). Dietary levels of carotenoid supplements range from 0.3-0.7%. Synthetic canthaxanthin containing 10% canthaxanthin if added to commercial feeds at 0.05% level would yield 5mg/kg feed.

Drugs/Antibiotics

They are used in feed to treat, cure, mitigate or prevent diseases. Recommended medicines for use in fish feeds are sulfamethzine, terramycin and furox. Oxalinic acid is used in feeds as antimicrobial drug in some countries of the world. Withdrawal period of residual medicines need to be recommended before sale of fish to consumers.

Hormones

Normally hormones are of three types; 1) those that affect growth and feed conversion, 2) those that affect sexual development 3) and those that affect osmoregulation. Addition of anabolic steroids and thyroid hormones increases appetite, FCR and growth. Pituitary growth hormones and insulin may also have growth promoting effects. In salmon over 90% increase in weight has been observed by feeding, 17 α-methyltestosterone at 1mg/kg of feed. Oral administration of T_3 (triiodothyronine) and T_4 (thyroxin) at 4-20 mg/kg of feed has significantly improved growth. Hormones are also used to induce sterility in fish because sterile individuals has faster growth rate than their normal counterparts under similar culture conditions. They are extensively used in tilapia for production of monosex population and to enhance its growth. Incorporation of androgenic steroids (ethyltestosterone and methyltestosterone) in diets (30-60mg/kg of feed) when fed for 14-21 days produces 90-100% male population. The synthetic androgen, 17α-methyl testosterone (MT) is used commonly in several countries for sex reversal of *O. mossambicus, O. niloticus* or *O. aureus*. Feeding lower doses of 17-β-methyltestosterone to rainbow trout and Atlantic salmon for 3 months resulted in all male population. In channel catfish they produced all female population, may be changed enzymatically to compound with estrogenic properties. Feeding estrogenic steroids to tilapia fry (ethynylestradiol, estrone, diethylstilbesterol) and salmon fry (17-β-estradiol) caused development of all females.

Antimicrobial agents

Diets containing more than 12% moisture can support bacterial, mold, and yeast growth unless they are stored frozen. Over 20 compounds are used but benzoates and parabens have wide spectrum antibacterial properties which are effective against bacteria, fungi, and yeast. Propionates are used primarily to inhibit yeasts and molds but are also effective against bacteria and fungi. Feeds need to be sealed in impermeable bags under vacuum with introduction of N_2 and CO_2. Moisture contents in feeds can be reduced with the addition of sugar, glycerol, NaCl and polypropylene glycol.

Anti-oxidants

Anti-oxidants are primarily used to prevent oxidation of lipids but other compounds like carotenoid pigments and tocopherols can also undergo oxidation. Auto oxidation, or atmospheric oxidation, produces products which induce off-flavors and off-odors in feed. Rate of oxidation depends on radiation levels, moisture, temperature, divalent cation concentration and O_2 concentration. Oxidation has three steps; initiation, propagation and finally termination. Antioxidants chelate divalent cation by acting as free radical acceptors. Two types of antioxidants are used, natural (vitamin E, C) and synthetic (BHA, BHT at 0.1% and ethoxyquin at 0.05%).

Attractants

Some materials with or without nutritive value are added to fish feeds to serve as attractants to enhance palatability. Squid meal, shrimp head meal or extracts improve feed acceptance to some penaeid shrimps. Fish meal is considered attractant in many fish feeds. Some chemical compounds such as free amino acids are highly olfactory and gustatory stimuli for fishes. Alanine and arginine which are highly taste effective to channel catfish signal the food source in environment but still remains to be determined. There are some synthetic compounds which serve as attractants but their efficacy still needs to be established (Hirt-Chabbert *et al*., 2011).

References

Aquaculture Development Coordination Program. 1980, Fish Feed Technology. Rome, FAO, ADCP/REP/80/11: 395 p

ADCP, 1983. Fish Feeds and Feeding in Developing Countries. United Nations Development Program and Food and Agriculture Organization of the United Nations, FAO, Rome. ADCP/REP/83/18:97p.

AQF 621, 2011. Fish Nutrition and Feed Technology, Agriculture Marketing Management Module by RUFORUM and Bunda College of Agriculture, Marketing University, Uganda P 37

Craig, S. and L.A. Helfrich., 2002. Understanding Fish Nutrition, Feeds and Feeding. Virginia Cooperative Extension, Publication 420-256. Pages 18.

Das, U.N., 2006. Essential fatty acids-a review. Current Pharmaceutical Biotechnology 7: 467-482.

Fitzsimmons, K., Dickenson, G., Brand, C., & Davis, J., 1997. Effects of reducing dietary lipid levels on growth and body composition of hybrid tilapia in an intensive recirculating-water system. The Progressive Fish Culturist, 59(4), 293-296.

Gatlin III, D.M. 2010. Principles of Nutrition. SRAC Publication No. 5003. Department of Wildlife and Fisheries Sciences, Texas A & M University, USA. Pages 8.

Halver, J. E., 2005. The Vitamins. In: Fish Nutrition, J.E. Halver and R.H. Hardy Eds. Academic Press Amsterdam, The Netherlands. PP. 62-132.

Hilton, J.W, C.Y. Cho, and S. J. Slinger., 1977. Factors affecting the stability of supplemental ascorbic acid in practical trout diets. J. Fish. Res. Board Can. 34: 683-687.

Hirt-Chabbert, J.A., A. Skalli, O.A. Young, and E. Gisbert, 2011. Effects of feeding stimulants on the feed consumption, growth, and survival at glass eel and elver stages in the European eel (*Anguilla anguilla*). Aquaculture Nutrition, 1-15.

Hublou, W.F., J. Wallis, L.B. McKee, D.K. Law, R.O. Sinnhuber, and T.C. Yu. 1959. Development of the Oregon pellet diet. Ore. Fish Comm., Res. Briefs, Corvallis, OR. 7: 28-56.

Jauncey, K., 1982 Carp (*Cyprinus carpio*) Nutrition - A review. In; J.F. Muir and R.J. Roberts (Eds.), Recent Advances in Aquaculture, London, Croom Helm, pp 217-63

Jauncey, K., 1982. The effects of varying dietary composition on mirror carp (*Cyprinus carpio*) maintained in thermal effluents and laboratory recycling systems. In: Proceedings of World Symposium on Aquaculture in heated effluent and Recirculation Systems, Vol.II, I.H. Heenemann Gmby and Co., Berlin.

Jauncey, K. and B. Ross, 1982. A guide to Tilapia feeds and feeding. Scotland Institute of Aquaculture, University of Sterling, 111 p.

Lall, S.P., 1981. Minerals -a Review, In Biological aspects of aquaculture nutrition. Proceedings of the World Conference on Aquaculture. Int. Trade Show, Sept. 20-23, 1981. Venice, Italy.

Lee, D.J. and Putnam, G.B., 1973. The response of rainbow trout to varying protein/energy ratios in a test diet. Journal of Nutrition 103: 916-922

Li, Z., W. Lei, J. Ye, and X. He., 1991. The nutritional value of commercial feed ingredients for Nile tilapia (*Oreochromis niloticus* L.) in China. In S.S. De Silva (Ed.), Fish Nutrition Research in Asia (pp. 101-106), Asian Fisheries Society Special Publication No. 5, Manila, Philippines.

Lim, C. and C.D. Webster, 2006. Tilapia: Biology Culture and Nutrition. The Haworth Press pp 530.

Lim, C., C.D. Webster and Cheng-Sheng Lee, 2008 Alternative Protein Sources in Aquaculture Diets, The Haworth Press, Taylor & Frances Group, New York & London. P 239, 286,

National Research Council, 1981. Nutrient Requirements of Fish. National Academy Press, Washington D.C.

National Research Council, 1993. Nutrient Requirements of Fish. National Academy Press, Washington D.C.

National Research Council, 2011. Nutrient Requirements of Fish and Shrimp. National Academy Press Washington D.C.

New, M.B, 1987. Feed and Feeding of Fish and Shrimp; A Manual on preparation and presentation of compound feeds for shrimp and fish in aquaculture. Aquaculture Development and Coordination Program, ADCP/REP/87/26., FAO, Rome. 275 p.

Rust, M.B, 2005. Nutritional Physiology. In: Fish Nutrition, J.E. Halver and R.H. Hardy Eds. Academic Press Amsterdam, The Netherlands. PP. 368-446.

Sargent, J.R., J.G. Bell, M.V. Bell, R.J. Henderson and D.R. Tocher, 1995. Requirement criteria for essential fatty acids. Journal of Applied Ichthyology, 11:183-198.

Sargent, J.R., D.R. Tocher, J.G. Bell, 2005. The Lipids. In: Fish Nutrition, J.E. Halver and R.H. Hardy Eds. Academic Press Amsterdam, The Netherlands. PP. 182-246.

Sedgwick, S.D., 1982. The salmon handbook. London, Andre Deutsch, 247 p.

Simopoulos, A. P., 1991. Omega-3 fatty acids in health and disease and in growth and development. The American Journal of Clinical Nutrition, 54(3), 438-463.

Sinnhuber, R.O., J.H. Wales, J.D. Hendricks, G.B. Putnam, J.E. Nixon, and N. E. Pawlowski, 1977. Trout bioassay of mycotoxins. In: J. VRodricks. C. W. Hesseltine and M.A. Mehlman (eds.).Mycotoxins in human and animal health. Pathodox Publishers, Inc., Park Forest South, IL.

Smith, R. R., 1976. Studies on the energy metabolism of cultured fishes. Dissertation, Cornell University.

Smith, L.S., 1980. Digestion in teleost fishes. In Lectures presented at the FAO/UNDP Training Course in Fish Feed Technology, ADCP/REP/80/11, pp. 3–17.

Takeuchi, T., S. Satoh, and W. Watanabe., 1983. Dietary lipid suitable for practical feed of *Tilapia nilotica*. Bulletin of the Japanese Society of Scientific Fisheries 49: 1361-1365.

Walker, S. and D. McMahon, 2008. Biochemistry Demystified. McGraw Hill, New York. 385 pages.

Webster, C.D., and R.T. Lovell, 1990. Response of striped bass larvae fed brine shrimp from different sources containing different fatty acid compositions. Aquaculture 90, 49-61.

Wirth, M., W. Steffens, T. Meinelt, and C. Steinberg, 1997. Significance of docosahexaenoic acid for rainbow trout (*Oncorhynchus mykiss*) larvae. Fett 99: 251-253.

Wilson, P.R., 2005. Amino acids and Proteins. In: Fish Nutrition, J.E. Halver and R.H. Hardy Eds. Academic Press Amsterdam Netherlands. PP. 144-175.

CHAPTER – V

FEEDING PRACTICES

Author
Prof. Dr. Muhammad Ashraf
Dean, Faculty of Fisheries and Wildlife
University of Animal and Veterinary Sciences (UVAS)
Pattoki Campus, Punjab - Pakistan.
E-mail: muhammad.ashraf@uvas.edu.pk

Co-author
Dr. R.S.N. Janjua
Country Representative,
American Soybean Association
World Initiative of Soy in Human Health
Karachi - Pakistan.
E-mail: janjua@soypak.org

Editor
Dr. Carlos A. Campabadal, Ph.D.
Outreach Specialist / Instructor in
Feed Manufacturing and Grain Storage
International Grains Program Institute
Department of Grain Science and Industry
Kansas State University
Manhattan, KS, U.S.A.

INTRODUCTION

The fundamental objective of feeding fish is to provide them a diet with the required nutrients for maximum growth, optimum yield, good health, and minimum waste, ultimately optimizing profits. Feed millers and farmers make choices to achieve these target parameters based on literature and his/her previous practices and experiences of feeding practices. These goals cannot be accomplished without provision of adequate quality feed which determines:

- Nutrient loading and water quality (and ultimately carrying capacity of fish) in the pond or other production system,
- Fish growth rate,
- Economic viability of the enterprise as 60-70% of variable production costs in fish culture projects in Pakistan are due to feed
- Health of the fish

Generally speaking fish feed can be divided into natural and artificial feed. Natural food principally comprises phyto and zooplanktons which are microscopic, and easy to ingest and digest due to the inherent presence of various enzymes which facilitate digestion of various nutrients present in feed. Natural food is preferably offered to larval fish of the cyprinid family due to their primitive stomach at hatching which is incapable of digesting inert feed particles prepared from a variety of ingredients. This food has its own merits and demerits; it is expensive to produce, normally contaminated due to the influx of unwanted organisms and sometimes contain toxic compounds deleterious for health and survival of the newly hatched larval fish In addition, its commercial production is not an easy task to cater to the demands of bigger fish or larger units of fish production. Consequently, the producer has to resort to artificial feed because supply is sustainable, it's easily available, storage is possible, and it's able to be manipulated as per requirements of different fish species and developmental stages.

Tilapia is a little different from other culturable fishes because larval Tilapia can easily accept and digest inert feeds. The plus point is that tilapia can equally feed on natural food and can grow efficiently if the feed is provided in adequate quantities. Artificial feed is provided to enhance productivity. For its intensive high production culture quality, artificial feed is of key importance for tilapia because it is a voracious feeder and accepts artificial much earlier and better than all commercially culturable fish species. Therefore, to enhance fish production per unit area and to keep the fish production units productive and sustainable, the provision of extruded pellets is inevitable for commercial tilapia production. However, the provision of feed for maximum production requires proper implementation of best management practices including well proven and accepted feeding practices which will be discussed in detail in the remaining part of this chapter.

FEED QUALITY

Feed quality refers to the nutritional efficiency and physical characteristics of the feed which makes it suitable for feeding and digestion in the typical fish digestive system. We set targets of different nutrients present in the right proportions to meet dietary requirements for good growth and keep up animal health for efficient performance (New, 1987). Presence of nutrients is a measure of feed quality but their assimilation into fish body weight is another integral parameter that affects fish performance. Nutrient requirements of fish vary with size, age, development and growth stage of fish and fish species because feed that is good for one species may not be equally effective in another species (Hasan, 2001). It becomes more obvious when two farmers culturing fish in two adjacent farms following similar protocols may not get equal fish production. Selection of quality feed ingredients and their blending in well-proportioned formulations, their processing (Hardy and Barrows, 2005) and ultimately safe and secure storage (New, 1987) before delivering to fish, are all critical steps to ensure quality. Regardless of how nutrient rich and balanced a feed formula is, it can work only if it is transformed into good physical product which means that physical attributes of feed play a decisive role in acceptability of feed and turning feed to fish and cash (Hardy and Barrows, 2005). The physical attributes of the feed determine the degree to which the feed affects water quality and consumption rates by the fish. The physical attributes of a good extruded feed are (New, 1987):

- Fine grinding of feed ingredients until they lose their integrity.
- Extruded feed must be fine free, too much fines means too much wastage, Tilapia can manage to eat these fines to some extent but most fishes cannot.
- Feed should be extruded pellet with water stability above ½ an hour.
- Feed pellets should preferably be floating with few exceptions. A majority of fishes readily take floating pellets which should be of uniform size and correspond to size of fish mouth; a size of about ¼ the gape of the mouth is advisable. It is much easier for the farmer to evaluate feeding response when using a floating feed. Nonetheless, floating feed often costs more. It is, therefore, up to the farmer to decide if floating feed is worth the added expense by evaluating fish performance and feed conversion.
- The feed should be palatable to the fish with a good taste, smell and feel.

One of the major differences in feeding fish and feeding live stock is that once fish have been fed, the excess feed cannot be retrieved from the water in pond, but can be in land animals if it is not contaminated with dirt or waste (Lovell, 1989).

In Pakistan, commercial fishes are cultured with both natural and formulated feeds so feeding methods differ and are adapted according to the particular fish species and individual farm pond ecological factors. Natural feed production is encouraged in ponds before introduction of fish and/or after stocking to support its daily consumption, natural food once produced is not stored and is available to fish immediately. Formulated, or manufactured feeds, can be stored for several months under cool dry conditions. The actual time before spoiling decreases if not properly handled and stored. Consequently it not only loses its nutritional quality but generates several deleterious products which can affect fish health (Goddard, 1996). This dictates that optimum handling and storage procedures for fish and/or shrimp feeds on farms, is an essential component of good feeding practices. Because formulated feeds come and are produced in many forms and shapes, they entail different handling and storage requirements.

STORAGE OF FEEDS

Since feed rations are composed of perishable nutrients, it follows that storage period should be kept to a minimum and that adequate storage conditions are provided to prevent deteriorative changes occurring in nutrient composition through oxidative damage and/or through microbial, insect or rodent infestation (McEllhiney, 1985). The most important environmental factors governing the storage or shelf life of feed ingredients and manufactured feeds are ambient temperature and humidity which dictates the pace of chemical changes and growth of contaminating pests such as fungi, bacteria and insects within the feed or the ingredients. Accordingly storage conditions and duration varies from feed to feed and its chemical composition.

FEEDS STORAGE

Aquaculture feeds, like most food products, have finite shelf life and special handling requirements. In order to realize full economic and nutritional value of feeds, it is necessary to store and handle them properly. For reasons of cost and convenience, dry diets are presently the most widely used feeds in aquaculture. These include extruded feeds, hard pellets, crumbles, and flakes. The general rule for preservation of these feeds is to store them in a dry, well-ventilated area that affords some protection from rapid changes in temperature. Cooler temperatures are best.

Storage times for dry feeds depend upon a number of factors:

- feed formulation,
- manufacturing methods and conditions,
- bag technology, and
- general storage conditions which are the most critical.

Diet type, the quality and quantity of fats are some other factors which contribute to feed integrity during storage. The quality of a feed begins to deteriorate immediately after manufacture. The rate and magnitude of decline can significantly be slowed, through proper feed handling and storage at the farm (McEllhiney, 1985). Extruded diets are commonly delivered to farms in 25 kg bags and rarely in 50 kg bags. In the future, big farms may utilize bulk delivery of feed using feeding trucks that connect through augers into feed silos. Bag or sack technology has evolved considerably in recent years, from paper and burlap, to coated paper, and plastic (polypropylene in many cases) bags. Paper bags are comparatively less expensive and accordingly provide the least protection. Proper storage becomes more detrimental if the extruded or pelleted feed is not properly dried and cooled before bagging. Formulated feeds with high oil contents are softer than conventional livestock feeds and hence more prone to damage if poorly handled (New, 1987). Farm staff should not be allowed to toss feed sacks carelessly or walk across the stacked sacks (Jukes, 1985). Stacks of 25 kg feed bags should not exceed 8-10 bags in order to avoid crushing of feed stacked at the bottom.

The potency of most vitamins contained in formulated feeds declines during storage. This is because many of these organic compounds are highly reactive and unstable. Under certain conditions they can be easily denatured by heat, oxygen, moisture and even ultraviolet light. The rate of vitamin activity loss in a given feed formulation is dependent on the particular vitamin, its source and the conditions under which feed is stored. Most manufacturing of aquaculture feeds recognize these potential losses. They attempt to fortify their diets with sufficient overages of each vitamin to provide the intended levels of activity within the declared product shelf life.

Always consider these guidelines during handling and storage of newly arrived feeds (Pedersen, 1985).

- Check the labels and buy the freshest feed in the store. Feed pelleted within the past 4 weeks often meets the nutritional and physical standards stated on the label. Feed degradation can include loss of vitamins, especially vitamin C, and an increase in mold, etc.
- Purchase only the quantity of feed that will be consumed within 4 to 6 weeks. Remember, the longer the feed is in storage, the lower its nutritional quality will be with time.
- During transportation and handling, protect the feed from moisture, heat and direct sunlight. Heat and sunlight directly destroy feed nutrients like vitamins.
- Store the feed in a cool, shaded, dry and well ventilated room. White, wooden buildings with reflective metal roofs are excellent for storing feed, but also metal sheds including metal containers can be helpful. Warm, moist and stagnant air enhances mold growth and attracts insects.
- Do not stack feed bags directly against a wall or on a concrete floor. Stack them on top of pallets at least 30 centimeters from the walls of the building to prevent moisture coming in contact

with the bags, easy quality inspection, and diminishes pest hideout places.

- Protect the feed from rodents, bats, chickens and other animals. The feed can be stored in cages made of wire mesh to keep off such animals.
- Try to minimize insect contact and infestation.
- Do not use or store pesticides or other toxic materials near the feeds.
- Do not keep feed that has been molded or spoiled. Learn what the normal color, smell and taste of the feed you use is. If the feed looks gray, blue or green in color; has a sour, musty or mildew odor (smell); or has been wet and has clusters of fused pellets - do not use it. Many fish are especially susceptible to mycotoxins.

NOTE: If you are feeding during the rain and the feed gets wet, feed all that wet feed that day or as soon as possible. Do not store wet feed, as it will get moldy and can contaminate dried feed.

FEEDING METHODS

Fish performance is not only dependent on feed pellet quality but also all fish should have access to the feed and at the same time it should keep in mind that no excess feed should be fed. Feeding fish correctly means;

- Giving feed of the correct nutritional quality for the specified age of fish,
- Feeding the right feed size for easy consumption,
- Feeding the correct amounts,
- Feeding at the right time(s) each day.

When fish are fed correctly, growth rates are good and uniform across the population, feed conversion ratios (FCRs) are low and pond water quality is better managed.

SELECTING THE TYPE OF FEED

Follow the following criteria for selection of feed:

- Different species have different nutritional requirements - follow the requirements for the species of fish being cultured.
- The age and size of the fish - Juvenile fish require higher protein in their feed. For grow-out production, a feed with a protein level of 32% is adequate for most aquatic animals.
- Select the highest quality feed.
- The anticipated feed conversion ratio (FCR) of the feed (Equation 1) - The cost effectiveness of the feed being used is governed by the FCRs obtained. For commercial grow-out ponds, FCRs should be less than 2:1.

ESTIMATING THE CORRECT AMOUNT TO FEED

In order to avoid over or under feeding the fish, the right amount of feed should be given each time. The amount of feed to be provided to the fish per day, and the feeding rate (ration), is dependent on the fish's body weight. Fish adjust their food consumption rates to meet their metabolic energy requirements (New, 1987). Therefore, the required ration varies with;

a) *The fish' size* (i.e. its average weight)
b) *The pond water quality* - temperature, dissolved oxygen and pollutant levels.

The amount of feed required per day (Feed Ration) can be estimated from feeding charts, nevertheless, it can be conveniently calculated on the fish production unit as given below;

> Total feed required per day (Feed Ration)
>
> = Sampled average fish size (weight) X feed rate (%) X total number of fish in the pond.

Or it can be expressed as

> Feed Ration
> = Av. Fish weight X feed rate (%) X total # of fish in pond

Where,
The feed rate is the amount recommended in the feeding charts (available in Fish Nutrition Books) as a percentage of the fish's average weight at that time (Azaza *et al*., 2009).

Example of Daily Feed Ration

i. If 5 g Tilapia requires ration of 8% of its body weight, how much food should it be given per day?

   Amount of feed to be fed per day = 5 g x 8/100 =0.4 grams feed per fish per day.

If there are 1000 fish in the pond, then; = 0.4 g x 1000 fish = 400 g of feed should be weighed out for the day.

ii. If 180 g Tilapia requires a ration of 2.5% of its body weight, how much food should it be given per day?

   Amount of feed to be fed per day (grams) = 180 grams x 2.5/100 = 4.5 grams feed to be fed per fish per day.

Accordingly for 1,000 fish = 4,500 grams feed is required per day.

According to the recommended feeding protocols, the fish should be receiving at least 2 meals a day. Therefore, divide 4.5 g by 2 = 2.25g. Feed about 2.25 g of feed per fish at each meal. As water temperatures are normally lower in the mornings, the fish may tend to eat less feed in the morning than they do in the afternoon. The optimum ration is the one that gives best growth rates, uniform growth and the optimum FCR (Feed Conversion Ratio). This is because at this level of feeding, there is minimum feed wastage and minimum deterioration of water quality. This is often achieved when fish are maintained at a feeding level just below that of '*satiation*'. DO NOT overfeed fish because it results in feed wastage, deterioration of water quality and subsequently poor growth. Overfeeding only serves to reduce profit margin. Likewise, substantial underfeeding results in poor growth and production.

ADJUSTING THE FEED RATION

Feeding rations should be adjusted either weekly or fortnightly depending on the fish's size. Fish size influences feeding rates. Smaller fish have a much higher metabolic rate and grow at a much faster rate so their rations need to be adjusted more frequently (preferably weekly). It is generally observed that younger or small fish require more energy for metabolism per unit body weight and have a faster growth rate than large or adult. Thus, smaller fish consume more feed on a percent weight basis than larger fish (Table 1). Water temperature is another highly significant variable that influences the metabolic rate and energy expenditure and has significant effects on the feed consumption and growth. The optimum temperature for growth of various species of tilapia has been reported to range from 26^0C to 32^0C. Tilapia is very sensitive to cold. They become generally inactive and feeding stops at about 16^0C and death may occur when exposed to temperature 12^0C for a few days, even though some species may be more tolerant than others (Ross 2000).

Feeding rations can be adjusted with the aid of feeding charts and occasional sampling (at least monthly) to ascertain actual fish sizes and growth rates. At sampling, adjust the ration based on the average weight of the fish obtained, NOT by the weeks in production. Fish do not feed at the same intensity every day. The amount of food they take in each day depends on the water quality on that day, notably the temperature and any stressors (low dissolved oxygen DO, high pH, high ammonia, disease, etc.) to which the fish are exposed (Santhanam *et al*., 1990). Feed rations should, therefore, also be adjusted on a daily basis. Therefore, on rainy cold days one may not feed fully, or at all, if fish show no interest in feeding as a result of lower water temperatures. Once a pond has reached its carrying capacity, stop feeding fish for growth but do feed a maintenance ration to prevent the fish from losing weight. Therefore, at carrying capacity a feed ration of about 0.5 to 1% body weight (depending on the fish size) is recommended (Lovell, 1989). Feed the smaller ration (0.5% body weight) to adult fish above 600 g (Table 1). Remember, when the pond's carrying capacity has been reached, fish will not grow regardless of how much you feed them. Feeding more at this time is wasteful. One would rather stop feeding all together if plan is to harvest or reduce densities by at least 30% of the pond total biomass within a week. After reducing pond biomass, water quality conditions improve in the pond, then start providing the full ration once again (Santhanam *et al*., 1990).

Table 1: Suggested feeding rates and feeding frequency for tilapia at different water temperatures

	Average body weight < 100 g		Average body weight > 100 g	
Water Temperature (^0C)	Feeding rate (%) of NR[a])	Feeding frequency (No. / day)	Feeding rate (%) of NR[a])	Feeding frequency (No. / day)
32 > t > 26	100	4	100	2 -3
26 > t >24	90	3	90	2
24 > t >22	70	2	60	2
22 > t >20	50	2	40	2
20 > t >18	30	1 – 2	20	1
18 > t >16	20	1	10	1
16 > t	No feeding		No feeding	

Source: Modified after Luquet (1991) *Note:* [a]Normal feeding rate.

ADMINISTERING THE FEED

The method of delivery of feed to fish plays a role in influencing growth rates, uniformity in size and FCRs. When administering the feed, we would like to see;

1. **Rapid and Complete Consumption of Feed by the Fish**

   This increases ingestion rates and ensures that pellets do not remain for a long time in the water before they are consumed. Otherwise, the pellets will fall apart, and nutrients will leach from the pellets into the water resulting in wastage of feed and worsening of water quality.

2. **Minimal Metabolic Energy Expenditure Associated with Feeding**

   Broadly spread the feed so that fish do not need to swim across the pond to get to the pellets. Also, broad distribution of the feed will lead to less jostling and energy expended to get at concentrated pellets. Feed the fish the largest particle size it can consume. For example, do not feed adult fish with powdered feed but rather with larger sized pellets. This allows the fish to utilize most of the energy they derive from the feed for growth, rather than for obtaining the feed. When fish are fed particles that are too small they end up spending a significant proportion of their energy trying to get enough food. Therefore, a 300 g fish should be fed a 5 mm pellet not a 1 mm pellet.

3. Ensure all the Fish have Equal Access to the Feed

When all fish have equal access to feed, more uniform growth rates are achieved and better FCRs are obtained. It is important to prevent a situation, where only a few fish dominate the access to the feeding area (Lovell, 1989). When this situation occurs, the aggressive fish get more feed and grow much larger and further increase the difference. Consequently overall survival rates and FCRs at harvest are reduced (New et al., 1997). The choice of farmer whether to feed fish by hand or whether to use labor saving mechanical or automatic systems depends on:

- Labor costs,
- The scale of farm operation,
- The species being farmed,
- The type of fish holding system, i.e. ponds, cages, or raceways, and
- Hatchery or grow-out operation

Keeping the above factors in mind following universally applied methods can be opted for feeding fish for maximum growth and minimum feed wastage (Goddard, 1996):

a. **By broadcasting** *(floating and sinking pellets)* Slow broadcasting of pellets is the recommended way for administering pellets to fish in grow-out ponds.

b. **Via automated and demand feeders** (floating and sinking pellets).

c. **Applied within feeding rings** (floating feeds – especially for juveniles in ponds)

When deciding what feeding technique to adopt, the following should be taken into account:

1. *How much feed should be fed per day per fish* (ration size)?
2. *How many times a day the fish should be fed* (i.e. the feeding frequency)?
3. *When the feeding times should be*? The amount of feed consumed and the rate at which fish can metabolize it depends on water quality. Therefore, avoid feeding early in the morning when water temperatures and oxygen levels are usually at their lowest.
4. *How you intend to administer the feed* (i.e., the feeding technique)?
5. *Labor availability and costs*.

COMMON FEEDING METHODS

1. *Hand Feeding*

Manual feeding allows close observation of the appetite and feeding behavior of those fishes which feed actively at the surface. Feed is sprinkled or broadcast on limited surface area, in response to the apparent demands of fish (Fig. 1). Problems of overfeeding can be resolved by gradual reduction in feed when fish approach satiation. Effective hand feeding can be achieved with experience and training. Fish appetite significantly fluctuates over time in response to physiological and environmental factors and hand feeding practice ensures that daily feed demands are catered accordingly and behavior of fish is monitored regularly. This method is labor intensive and time consuming hence its applications may be limited on large farms. Despite these limitations at least 20% of the total feeding should rest with manual feeding for close and regular monitoring of fish condition and adjusting ration size accordingly.

Fig. 1 a. Hand Feeding of Fish in cages

Fig. 1 b. Broadcasting feed in tilapia fish pond at Duya pur,Multan dated August 27, 2014. Photo courtesy: R.S.N. Janjua, SoyPak (ASA/WISHH)

II: ***Demand Feeders***

These are also referred to as response feeders, most commonly comprise feed hoppers fitted with pendulum and feed release valve. The pendulum is attached to some type of valve mechanism that controls flow of food from the hopper. When fish bite or hit the pendulum, a controlled quantity of food is released into the water. This demand feeder is most appropriate for bigger fish as small fishes typically do not have sufficient body mass to affect the mechanics. A different demand feeder is used for small fish where the feeder has a submerged feed tray linked to hopper and releases food when fish eats all the feed present in tray. Advantages and disadvantages of this system are not yet fully established (Goddard, 1996).

Fig. 2 Demand feeder (Source Pond King Inc. 2015)

Demand feeding systems are also available which link a touch sensitive probe through a control system to an automated feed dispenser. When activated by fish the feeder dispenses a predetermined amount of food from a hopper by a vibrator or food spreader (Fig. 2). These systems offer better feed distribution than conventional demand feeders. Conventional demand feeders have limited applications in open windy sites where water waves and wind action triggers pendulum movement and may allow feed to flow freely. Demand feeders are cheap to buy and/or simple to construct and do not need external energy sources to operate. They are available in wide range of sizes and feed holding capacities (Tacon, 1990).

III: ***Automatic Feeders***

These dispense pre-determined amounts of feed at set intervals and vary widely in the sophistication of their design and operation. Most of them are electrically powered from batteries, solar cells or from the power grid and may be controlled by a central control unit (Fig.3).

Fig. 3 Automatic Feeder (Source Pond King Inc. 2015)

Feed may be spread as it falls or is broadcast from the hopper by blowers or revolving or vibrating mechanisms in circular or semicircular patterns. They may be positioned centrally or on a side of the fish farm unit. These systems can also distribute feed through pipes from central storage silos or mixing units. The timing and quantities of feed delivered to holding units are controlled by computerized systems capable of integrating environmental information (water temperature and light conditions), with fish biomass data (fish numbers and average weights) to calculate, control, and record feed quantities and feeding frequency. Water flow and compressed air can be used to dispense feed in cages present near shores.

IV. *Hatchery Feeders*

Feeding systems in hatcheries must be capable of delivering feed almost continuously since small fish are growing rapidly and have high metabolic demands. Hatchery feeds are generally the most expensive type but are used in far less quantities. Hatchery feeds are finely powdered or crumbled and may contain large proportions of oil, hence they may be sticky with poor flow characteristics and has a bearing on the design of the feeder. Many types incorporate a revolving disc or plate on which food is placed, or fed from hopper. As the plate slowly revolves, food is pushed from the plate by a radial arm and falls into the tank below. By regulating the speed of rotation of the disc, the quantity of feed dispensed can be controlled reasonably accurately. Another popular type of hatchery feeder incorporates a clockwork driven moving belt (Goddard, 1996). As the belt moves slowly forward, food falls from the edge of the belt through a wide opening into the tank below. These feeders are cheap and easy to construct and operate efficiently in damp hatchery conditions (Fig. 4). The clockwork mechanism is rewound manually by pulling the belt back into place.

Fig. 4 Auto Pond Fish Food Feeder with Digital Timer
(Source Pond King Inc. 2015)

V. *Mobile Feeders*

Mobile feeders combine some of the advantages of hand feeding with the labor saving benefits of automated feeders. They can be mounted on trucks or boats or can be connected to the power takeoff of farm tractors to operate powerful blowers. Mobile feeders come in a wide variety of sizes and shapes and most of them can be operated by a single person.

VI. *Sub-surface Feeders*

A novel approach to avoiding feed waste in cage rearing systems is that of releasing extruded floating feed from devices at the bottom of cages. Feed of selected density, released from the floor of the cage, slowly floats upward through the cage toward the surface. Uneaten feed that comes to the surface indicates the need to reduce or stop the feeding. It reduces up and down fish migrations for feeding and gives them more time for feed ingestion (Fig. 5).

Fig. 5 Sub-surface Fish Feeder (Source Pond King Inc. 2015)

FEEDING FREQUENCY

The feeding frequency is the number of times fish in a pond are fed in a day. (Table 2). The feeding frequency affects the efficiency of feed utilization (i.e. the FCR) so it is important to establish the optimal frequency of feeding to attain the best possible (optimal) FCR and uniform sizes of fish. The following points should be taken into account, when deciding how frequently fish should be fed each day (New et al., 1997):

1. For optimum growth and feed conversion, each feeding should be about 1% body weight. However, it is expensive in terms of labor to feed 4 or 5 times per day. In grow-out ponds, feeding 2 or 3 times a day is adequate.
2. Proper feeding frequencies reduce starvation and result in more uniform sizes.
3. Juvenile fish need to be fed more frequently than adults, because they have higher metabolic rates and their stomachs are too small to hold all the feed they require for a day.
4. Fish from 400 g can be fed once a day, because at this size the stomach can hold enough food for the day. At this stage, feeding all the fish at the same time once a day, results in more uniform growth rates because the greedy ones will be full when there is still feed around in the pond. This provides a good chance for the smaller fish to come and feed, hence, they also grow.
5. The feed administered at a meal should be consumed within the first 15 minutes of the feeding if floating feed is administered. If it is not, reduce the amount given to match how much can actually be consumed during this period. This is a bit tricky with sinking feed but it is possible.

Table 2. Suggested daily feeding rate and frequency for various sizes of tilapia at 28^{0}C.

Fish Size	Feeding rate (percent of body weight)	Feeding frequency (No. of times / day)
2 days old to 1 g	30 - 10	8
1 - 5 g	10 - 6	6
5 - 20 g	6 - 4	4
20 - 00 g	4 - 3	3 - 4
> 100 g	2 - 3	2 -

Source: Modified after Lim (1989)

FEEDING RESPONSE

It is important to closely watch the response of fish to feed for daily feeding and planning the quality and quantity for the days ahead (Santhanam *et al*., 1990). Fish response to feed is a useful observation because;

1. It enables the farmer to feed the fish based on their actual needs at each meal. Therefore, the likelihood of overfeeding or underfeeding is reduced to a minimum.
2. It enables the farmer to visually assess the number of fish in the pond, and their growth on a daily basis without actually having to physically sample and handle the fish. The only time a farmer can see most of the fish in the pond in bulk is during the course of feeding, that's why, feeding by response also provides another avenue for inventory control.

3. When water quality conditions in the pond are poor, or fish are sick, their first response is to go off feed. When fish are fed by response, it is easy to detect when they have lost their appetite. Therefore, problems can be detected sooner, and remedial measures should be affected promptly. The fishes' feeding response, is therefore, the first indicator of the fishes' well being.

The fish's feeding response depends on the:

1. *Suitability of the Feed*. The feed's appearance, smell, texture/feel and taste influence the fish's appetite. The more palatable the feed is, the better the feed response should be.
2. *Culture (Water) Environment*. The most important water quality parameters that affect feeding response in ponds are water temperature and dissolved oxygen. The warmer the water and more dissolved oxygen it has, the more active fish will be with better feed consumption and FCR.
3. Other stressors, such as pollutants in water, other water quality variables (notably of ammonia and pH), handling and social interactions also affect the fish's appetite (Santhanam *et al*., 1990). When fish are stressed, their appetite drops quickly.

ASSESSING FEEDING RESPONSE

Examination by farmer during feeding is extremely important in assessing how much the fish actually need to be fed at each meal, or that day. In order to make this assessment, the following should be noted by the farmer during feeding:

- How fast the fish moves towards the feed and how this reaction/behavior can be compared with that at previous feedings?
- Whether or not the fish are interested in the feed?

- What the color of the pond water is prior to feeding?
- What proportion of the fish comes to the feed?
- What the weather was a few days before, and on that day? Is (was) it rainy, cold or hot?

  Therefore, the farmer must always stay around during feeding to observe how the fish feeds every single day. Simply calculating and feeding the amount prescribed by the feeding chart results in wastage, higher FCRs and poorer water quality. Feeding based on calculations only, is therefore "dumping" the feed, or "feeding the pond"; not feeding the fish (New, 1987).

GROWTH PARAMETERS AND DIGESTIBILITY

Growth performance of the fish can be evaluated by the following mathematical expressions (Azaza *et al*., 2009).

Weight gain %

$$= \frac{\text{Final body weight} - \text{initial body weight}}{\text{Initial body weight}} \times 100$$

Specific growth rate (SGR)

$$= \frac{\ln \text{Final body weight} - \ln \text{Initial body weight}}{\text{No. of days}} \times 100$$

Feed conversion ratio (FCR)

$$= \frac{\text{Feed consumed}}{\text{Weight gained}}$$

Feeding rate

$$= \frac{\text{Feed consumption} \times \text{No. of days}}{\text{Weight gain}}$$

Assimilation

$$= \frac{\text{Feed consumption}}{\text{Fecal output}}$$

Protein efficiency ratio (PER)

$$= \frac{\text{Weight gain}}{\text{Assimilation}}$$

Apparent protein digestibility (APD)

$$= \frac{\text{Assimilation x 100}}{\text{Food consumption}}$$

Criteria for Judging Feeding Response

The following is a description of the criteria used to judge the fishes' feeding response:

E – Excellent – Fishes are very active and come to feed immediately. The feed administered is all consumed by the fish within 5 to at the most 10 minutes of feeding.

G – Good – Fishes are less active and come to feed over a longer duration. Feed gets consumed in about 15 to 20 minutes.

F – Fair – Fishes are sluggish but do consume about three quarters of the feed. However, they do so in more than 30 minutes.

P – Poor – When feed is applied, fish do not come to feed. More than three quarters of the feed administered is left over.

These criteria may vary from fish to fish, farm to farm or farmer's personal experiences and observations. As much as possible, the same person should feed the fish on a daily basis. Likewise, the person who feeds the fish should be the one who keeps the daily feeding records, not someone else.

Training Fish to Feed by Response

Fish should be trained to come up, and get their feed at the water surface (New *et al*., 1997). In order to do this, the following steps should be followed when fish are fed by the slow broadcasting technique:

a. ***Administer the feed at the same place in the pond and at about the same time every day***

This gets the fish into the habit of being in a certain area of the pond at feeding time. If the fishes do not come to the area to feed initially, do not add any more feed until they learn to come to the assigned feeding area. It may take up to a week to train fish to come and feed from the same area and learn their feeding times. Do not worry if in the mean time they do not get much. One may stop at the edge of the pond, to call the fish at feeding time before administering the feed to them. Clapping or banging on bucket or stamping feet, will signal fish that feed is about to distributed. Again, it may take a week or two for the fish to learn the signal.

b. ***Broadcast a handful or plate full of the feed once most of the fish have collected at the feeding area***

If the fish come out to get the feed and immediately consume the 'tester', then the rest of the feed may be added. However, do not trickle the rest of the feed into the pond bit-by-bit. Rather, steadily broadcast large scoopfuls or bucketfuls at a time, until the fish's response starts to slow and the fish shows no more interest in coming back for more feed. Weigh any leftover feed and keep it for the next meal (Tacon, 1990). By training fish to feed in this way, one is deliberately creating competition for food. The fishes soon realize that if they do not come to feed at meal times, then they will not have food until the next meal time. Therefore, the fish actively compete to get to the feed at meal times and eat as much as they can, as fast as they can. Because all the fish eat at the same time, growth rates become more uniform and FCRs consequently improve.

When Not to Feed Fish

1. ***The Feeding Response is Poor***

When the fish show a poor feeding response, it is normally for a reason - the water quality may have changed, or on a cold wet day, the pond water temperature may have dropped. Therefore, do not add more food than the fish are interested in consuming. Rather, find out the cause of the poor response and if it is due to something that can be mended, then correct it.

2. ***They are Feeling Unwell***

When fish are sick, they go off feed. If someone insists on feeding them, they still will not eat. The feed administered will instead accumulate at the sides or the bottom of the pond, and cause the water quality to drop. No positive returns accrue from wasted feed. Instead losses build up due to reduced water quality, higher FCRs and the lost income from the wasted feed.

3. ***Two Days before Harvest or Transportation***

This is to allow them to empty their guts before harvest or transportation. In doing so, water quality in transport containers can be better maintained and stress levels during transportation can be minimized. The other objective is to improve quality of the harvested product for the market and reduce volume of offal in the processing plant.

4. *The Afternoon before Sampling and on the Sampling Day before initiation of Sampling*

Fish should not be fed the afternoon before sampling and also do not feed them on the actual day of sampling especially before they are sampled. This is because they will be subjected to a lot of stress from physical handling during seining, weighing and counting. In addition, the act of passing a seine through the pond has a sequential negative effect on water quality because of the mixing of the top and bottom pond water. The bottom water is often of poorer quality. Young fish still being fed more than once a day, may be fed that day after sampling at their normal feeding time. Because of the stressors the fishes have been exposed to at sampling, their feeding response is likely to be poor for up to two days after sampling. The same situation will occur with partial harvests because the fish will still be recovering from the handling stress. Therefore, do not insist on giving the fish their full ration, if they show no interest in feeding. Only give the full ration when their response picks up (Santhanam *et al*., 1990).

5. *When Treatments are applied to the Pond*

When some treatments like formalin or algae controls are applied to the pond, the fish may get stressed because the water quality within the pond has been altered. Their appetite subsequently drops. It is best to allow the water quality to improve and when it does, so will the fishes feeding response improve.

6. *When Water Temperatures are Low on Rainy Days*

After a series of rainy days if the water temperatures drop below 22 ^{0}C (Bhatnagar, and Devi, 2013), the fish are unlikely to be interested in feeding, therefore, do not feed. This can occur during the winter or summer monsoons in Pakistan.

EVALUATING FEED PERFORMANCE

Feed is the input with the greatest influence on water quality during production. Feed is also the input with the highest cost during the course of production. Feed performance alone, can therefore single handedly make or break one's business. Therefore, it is extremely important to closely monitor the performance of feeding during the course of production, in feed-based systems.

FEEDING RECORDS

Records of feed usage should indicate:

1. *The type of feed(s) administered*,
2. *The amount of feed given each day*,
3. *The feeding response at each feeding*,

Feeding Records will help assess cost-effectiveness of the feeding program.

FEED CONVERSION RATIO (FCR)

The Feed Conversion Ratio (FCR) as previous explained, is the amount of feed required to produce a unit of fish and is expressed mathematically as below in the equation 1.

Equation 1

$$FCR = \frac{\text{Total amount of feed fed}}{\text{Total weight of fish harvested}}$$

The indicators of FCR are;

i. *Performance of a feed*,

ii. *Performance of the person feeding the fish and the fishes' health,*

iii. *Cost-effectiveness of using a particular feed.*

Example on Calculation of FCR

If total 5,000 kg feed is offered to fish to produce 4,000 Kg of fish biomass at harvest (and initial stocking weight of fish is 10 kg then as per equation 1;

$$FCR = \frac{5{,}000\ kg}{(4{,}000\ kg - 50\ Kg)}$$

$$= 1.26$$

This means a total of 1.26 kg of feed will be used to produce each kilogram of fish.

Cost of feed required for one kilogram of tilapia.

On the basis above given data, if each kilogram of feed costs Pak. Rs. 70.00 then at FRC 1.26 the total feed cost is calculated below;

Cost of Feed = FCR x cost of feed Rs / Kg

= 1.26 x 70

= Pak Rupee 88.20

In grow-out operations, a good FCR should be between 1.2 to 2 when using the extruded pellets currently available in the market. The FCR should never be above 2 (Craig and Helfrich. 2002.

Possible reasons for poor FRC

i) *Poor quality feed is fed*. This occurs when feed is of poor nutritional value or the pellet is of poor physical quality. It is common to have high (poor) FCR rates when mash is fed to the fish.

ii) *The feed (size or nutritional quality) given is not suitable for the age of fish being grown.* For example, the pellet may either be too big or too small, or contains nutrients in the wrong proportion, etc.

iii) *The culture conditions are stressful to the fish.* For example, if dissolved oxygen levels are continuously below 1 mg/L and/or total ammonia N levels are high (>8.5-20 mg/L) (Brune and Tomasso, 1991), as commonly occurs when ponds have attained their carrying capacity.

iv) *Fish are 'over-fed'*.

v) *Survival rates at harvest are low*. Low survival rates may arise as a result of stocking small sizes, poor handling at stocking, predation, etc.

vi) *Feeding for growth when the pond is at its carrying capacity*.

How to Assess the Cost-Effectiveness of a Feed

When evaluating the cost-effectiveness of a particular feed, the FCR of that feed and its unit cost should be taken into account simultaneously and not independently of each other. Using the cheapest feed available, more often than not, does not translate into the lowest cost to produce a kilogram of fish. It will be of worth mentioning that quality feed dictates the quality of water. Better quality of water supports high stocking densities consequently producing more fish in a given fish farming unit moving towards intensification saving water and other overhead expenses (Tacon, 1990) (Table 3).

Table 3. Cost comparison of different representative fish feeds offered to Tilapia.

	Rice/Maize bran	**Farm mixed fish feed mash**	**Complete diet (extruded floating pellets)**
Unit Cost of feed/kg	Pak Rs. 28/-	Pak. Rs.38/-	Pak Rs. 70/-
FCR of the feed	9	5	1.26
Total Cost (Pak Rs.) of feed used to produce a kilo of fish	= 9 kg bran x Rs.28/ kg = Rs. 252/-	5 kg feed x Rs. 38/ kg = Rs. 190/-	= 1.26 kg pellets x Rs. 70/kg = Rs. 88.2/-

Note: Values of FCR varies with types of fish cultured and location of the fish culture unit as well as cost of feed and its physical nature and chemical composition.

Commonly used Farmer's home-mixed feed is comprised of 50% Rice/maize bran, 5% fish meal, 20% sunflower meal, 20% cotton seed meal and 5% molasses.

The lower the FCR, the less amount of feed used to produce a kilogram of fish. Therefore, the feed which gives the lowest FCR, even though it might be more costly, is often the one that gives the lowest cost of production and also enhances the productivity in ponds. (Chapter 12).

MANAGING FCRS

Ensuring that FCR remains within an economic range (i.e. of not more than 2 at harvest), is extremely important when raising fish using 'feed based' technologies. An FCR greater than 2, more often than not results in economic losses. This is because about 70% of one's operational costs are spent on buying feed for the fish. So any slight drop in the FCR, results in a significant increase of one's profit margins. The FCR obtained is simultaneously influenced by the *quality of feed given*, *the fish themselves*, *pond water quality* and *feeding management*. These factors act together and determine the fish's appetite, as well as how much of the feed eaten is actually digested and used for growth. Hence, they collectively determine what the FCR shall be at any given time. All four factors must perform optimally to get an optimum FCR (New et al., 1997). A lapse in any one of four results in higher FCRs.

Table 4. Proportionate Change in Cost Structure of various components for Major Production Inputs over the Course of Production.

	% Total Variable Costs		
Time(days)	Fingerlings	Feed	Labour
0	98.7	0.0	1.3
18	91.5	6.7	1.8
28	1.4	16.1	2.5
48	66.7	29.8	3.5
61	55.1	41.2	3.7
83	46	50	4.2
117	38	57	5
175	32	62	6
208	29	65	6.5
283	24	69	7.3
311	23	70	8

(New, 1987)

Above table shows how much contribution is of feed in total expenses incurred on fish production, it is therefore, important to use feed as efficiently as possible in order to optimize returns (Table 4). The FCR is dependent on four major factors; feed, person feeding fish, water quality and the fish. Therefore,

1. **The person feeding the fish** is highly important and he/she should be in a position to:

i. Train fish to respond to feeding times.
ii. Keep track of and evaluate fish feeding response as well as fish performance through actual observation and keeping of records (i.e., with quantitative as well as qualitative information).
iii. Keep track of fish numbers and sizes in the various production units during the course of production.
iv. Deduce correctly from the pond and feeding records as well as the fish's feeding behavior, what the next course of action should be (e.g. what type of feed to give, how much feed to give, whether or not to adjust or withhold feeding, how best to administer the feed, what pond/water management details need adjusting, etc.). If the person feeding cannot do this, then it is not worth spending money on commercial feeds as you will end up losing money instead.

2. The Feed

i. Quality (both physical and nutritional). Having a well made pellet of the correct size and of the right nutrient value for the size of fish being reared, is extremely important. Pellet integrity is also important.
ii. Quantity. It is important that the right amounts are fed.

3. The Fish

i. The species being raised. For example, tilapia fingerlings will perform better than other commercially cultured fishes in an earthen pond receiving only fertilizer as an input.
ii. The size of fish. Fry require a higher protein level in their feed as well as a smaller feed pellet compared to adult fish.

iii. Quality of seed stocked. For example, was the fish stressed at stocking? Was it of the correct stocking size for the unit and intention for which it is being raised? For example, Tilapia grow out ponds should be stocked with fish of not less than 5 g but preferably with fish of 10 g and above (Hardy and Barrows, 2005). Nursery ponds on the other hand, are managed to ensure survival of young fry and can therefore be stocked with fish of 1-5 g.

4. Water Quality within the Production Pond

i. the water temperature,
ii. levels of oxygen,
iii. levels of ammonia, pH, and pollutants in water.

MANAGING FISH WASTES

The most important rule in fish nutrition is to avoid overfeeding, which is a waste of expensive feed. Overfeeding also results in water pollution, low dissolved oxygen levels, increased biological oxygen demand, and increased bacterial loads. Usually, fish should be fed only the amount of feed that they can consume quickly (in less than 15 minutes). Many growers can take advantage of the floating (extruded) feeds, based on the type of fish they have at their farms, in order to observe feeding activity which helps to judge if feed given is more than required or less than the fish actually need. However, the type of feed to be fed to fish varies from species to species; some may prefer sinking feed which makes it hard to assess the quantity of feed consumed. Administration of floating feed and assessment of its consumption status is applicable to only those species which accept floating feeds.

Even with careful management, some feed ends up as waste. For example, out of 100 units of feed given to fish, typically about 10 units of feed are uneaten (wasted) and 10 units of solid and 30 units of liquid waste (50% total wastes) are produced by fish. Of the remaining feed, about 25% is used for growth and another 25% is used for metabolism (heat energy for life processes) (Craig and Helfrich, 2002). These numbers may vary greatly with species, sizes, activity, water temperature, and other environmental conditions.

Summary Guidelines for Feeding Extruded Pellets

Besides the quality of the fish stocked, feed is the most important input in pond/tank fish because (New et al., 1997):

1. Feed is the highest proportion of operational costs and, therefore, the profitability of the operation depends largely on the performance of a feed (i.e. FCR). Remember, the aim is to convert the feed into fish to sell (Tacon, 1990).
2. Pond production performance is attributable to the feed quality and the feeding technique.
3. Using a feeding technique based on feeding response is the best way a farmer can keep track of the:
   i. Number and size of fish in the pond between samplings and at harvest
   ii. Health status of the fish

In order to get the best out of a feed, one must (Tidwell, 2012);

1. Construct and prepare ponds for convenient culture of fish.

2. Stock the ponds based on their carrying capacity in relation to targeted harvest size.

3. Ensure best water quality.

4. Feed the best quality feed available and always aim for better fish production with minimum feed provisions and lower FCR values which is possible following these rules;

   a. Feed the right feed correctly based on the fish's feeding requirements and response.

   b. Be conservative when using feed because it costs money.

   c. The feed used must match the pond's inventory. Know the numbers and sizes of fish in the pond. Adopting a single batch system of management (stock one size and harvest all before re-stocking pond) allows better knowledge of what is actually in the pond and the population's size distribution. This is extremely important in production because in some species, the larger fish may predate upon the small fish.

   d. Avoid overfeeding. One would rather keep fish slightly hungry than overfed.

   e. Avoid swings in feed input i.e. impromptu or haphazard feeding. Other than increasing FCRs such feeding results in increased size variation. Fish lose weight fast when not fed for a while.

   f. Base your feeding rate on the fish's feeding response using the feeding chart only as a guide. Feeding by response means the person feeding MUST take time to feed and observe how the fish are feeding.

5. Keep and regularly evaluate pond and feeding records. The person responsible for feeding should keep the daily feeding records. Adjust pond management and feeding based on the information derived from the records.

6. Harvest the production ponds before they reach carrying capacity. In the event that it is not possible to harvest the pond or reduce the fish density,

   a. 'Flush' water through the pond before it gets to carrying capacity when there are signs of water quality deterioration, and

   b. Feed only a maintenance ration: about 0.5% to 1% body weight per day.

SUMMARY

Fish food comprises both natural and artificial feeds. Choice of feed depends on the nature of fish species and development stage of fish. The majority of fish species do not accept artificial feed at larval stage and natural food has to be provided for their survival and reasonable growth. At later stages and higher stocking densities natural food cannot fulfill the fish requirements and farmer has to resort to artificial feed. Tilapia, however, can accept artificial feed soon after hatching. Commercial culture of conventional fish species is impossible without inclusion of artificial feed in one way or the other. There are several feed formulations, forms of feed and a variety of feeding methods. Farmer can adopt any of them according to availability of feed formulas and type of feed keeping in mind the type of fish species cultured and his economic status. Merits and demerits of natural and artificial feeds, storage, formulations and feeding methods, as well as ways and means to get good feed conversions and better productions, have been discussed.

References

Azaza, M.S., W. Kammoun, A. Abdelmouleh, M. M. Kraı¨em, 2009. Growth performance, feed utilization, and body composition of Nile tilapia (*Oreochromis niloticus L.*) fed with differently heated soybean-meal-based diets. Aquacult Int., 17:507–521.

International Journal of Environmental Sciences Volume 3, No 6, 2013 © Copyright by the authors - Licensee IPA- Under Creative Commons license 3.0

Research article ISSN 0976 – 4402 Received on March 2013 Published on June 2013

Bhatnagar, A. and Devi, P., 2013. Water quality guidelines for the management of pond fish culture. International Journal of Environmental Sciences, 3(6): 1980-2009.

Brune, D.E. and J.R. Tomasso, 1991. Aquaculture and Water Quality. Published by the World Aquaculture Society USA. Pages 606.

Craig, S. and L.A. Helfrich, 2002. Understanding Fish Nutrition, Feeds and Feeding. Virginia Cooperative Extension, Publication 420-256. Pages 18.

Craig, S., 2009. Virginia Cooperative Extension; Understanding Fish Nutrition, Feeds, and Feeding, Virginia-Maryland College of Veterinary Medicine, Virginia Tech Louis A. Helfrich, Extension, Department of Fisheries and Wildlife Sciences, Virginia Tech Publication No. 420-456, Pages 4.

Hardy, R.W. and F.T. Barrows., 2005. Diet Formulation and Manufacture PP 506-596. In: Fish Nutrition, J.E. Halver and R.W.Hardy Editors, Academic Press Amsterdam, The Netherlands.

Hasan, M.R., 2001. Nutrition and Feeding for Sustainable Aquaculture Development in the Third Millenium. In: Technical Proceedings of the Conference on Aquaculture in the Third Millenium, R.P. Subasinghe, P.B. Bueno, M.J. Phillips, C. Hough, S.E. McGladdery and J.R. Arthur Editors. Pages 471.

Goddard, S., 1996. Feed Management in Intensive Aquaculture. Chapman & Hall New York. Pages 194.

Jukes, S., 1985. Control of Fire and Explosion in Feed Plants. In: Feed Manufacturing Technology III. R.R. McEllhiney Technical Editor, American Feed Industry Association, Inc. 1701 North Fort Myer Drive, Arlington Virginia 22209. Pages 608.

Lim, C., 1989. Practical feeds-tilapias. Pp . 163-167 in Nutrition and Feeding of Fish, R.T. Lovell, ed. New York: Van Nostrand Reinhold.

Lovell, T., 1989. Nutrition and Feeding of Fish. An AVI Book, Van Nostrand Reinhold, New York. Pages 260.

Luquet, P., 1991. Tilapia Oreochromis spp. Pp 169-180, in Handbook on Nutrient Requirements of Finfish, R.P. Wilson, ed. Boca Raton , Fla:CRC Press

McEllhiney, R.R., 1985. Feed Manufacturing Technology III. American Feed Industry Association, Inc. 1701 North Fort Myer Drive, Arlington Virginia 22209. Pp 608.

Pedersen, J.R., 1985. Sanitation and Pest Management. In: Feed Manufacturing Technology III. American Feed Industry Association, Inc. 1701 North Fort Myer Drive, Arlington Virginia 22209. pp.380-389.

Pond King Inc. 5924 W. Hwy.82-Gainesville, TX 76240, USA, Phone 940.668.2573.

New, M.B., 1987. Feed and Feeding of Fish and Shrimp. UNDP, FAO of the United Nations, Rome, 1987. 275 pages.

New, M.B., A.G.J. Tacon, and I. Csavas. 1997. Farm-made aquafeeds. Daya Publishing House Delhi. Pages 434.

Santhanam, R., N. Sukumaran and P. Natarajan., 1990. A manual of Fresh-water Aquaculture. Mohan Primlani for Oxford and IBH Publishing Co. Pvt. Ltd. 66 Janpath New Delhi. Pages 193.

Tacon, A.G.J., 1990. Standard Methods for Nutrition and Feeding of Farmed Fish and Shrimp. Argent Laboratories Press USA. Pages 208.

Tidwell, J.H., 2012. Aquaculture Production Systems. World Aquaculture Society, Published by Wiley-Blackwell, John Wiley and Sons, Inc. 222 Rosewood Drive, Danvers, MA, USA. Pages 421.

CHAPTER – VI A

THE SOYBEAN AND ITS PRODUCTS

Author
R.S.N. Janjua
Country Representative,
American Soybean Association
World Initiative for Soy in Human Health
Karachi – Pakistan
E-mail: janjua@soypak.org

Editor
Karen L. Veverica
Director, E.W. Shell Fisheries Center
School of Fisheries, Aquaculture and Aquatic Sciences
Auburn University, AL USA

1. INTRODUCTION TO SOY AND SOY PRODUCTS

Soybeans (US) or Soya bean (UK) is a species of legume that originated in China and has been used in East Asia for more than three millennia. More recently, it has been has been successfully cultivated around the world. The plant is classed as an oilseed rather than a pulse by the UN Food and Agriculture Organization. Soybean is a highly versatile crop and has many uses but it has become a major source of protein-rich feed ingredient for diets used in the poultry, swine and aquaculture industries throughout the world.

The soybean plant [Glycine max (L.) Merrill], is bushy with height ranging from 0.75 – 1.25 m that branches sparsely and densely depending on cultivators and growing conditions. The pods, stems and trifoliate leaves (sometimes with 5 leaflets) are covered with fine brown or gray pubescence. Soybean crops can build soil fertility by fixing large amount of atmospheric nitrogen using bacteria located in their root nodules.

There are over 2,500 varieties of soybeans that vary greatly in size, shape and color. Shapes vary from flat to spherical and colors range from yellow to green, brown and black. Modern varieties, mainly grown for their oil content, are generally spherical in shape with yellow or green as the accepted seed coats.

The soybean consists of two cotyledons which represent approximately 90% of the weight, a seed coat or hull (8% of weight), and two much smaller and lighter structures the hypocotyls and the plumula. The cotyledons contain the proteins and lipids (oils) that constitute the main nutritional components of the soybean products obtained from soybeans. They are also the main storage area for the carbohydrates and various other components of importance, most notably the enzymes (lipoxygenase, urease) and the anti-nutritional factors (ANF).

Advances in soy processing have allowed for the increased use of soy in both human and animal nutrition following proper treatment or extraction of the soybeans to eliminate many of the anti-nutritional factors (ANF). Soy is one of the few plants that provide a complete protein as it contains all eight amino acids essential for human health. The digestibility of the protein fraction is high and the amino acid profile, when matched with a grain provides a close match to animal requirements. For this reason, and because of the higher amount of protein produced by soybeans per unit area of land compared to any other crop, it has been called the **"wonder bean"** and **"nature's miracle bean".**

1.1. Production, Consumption and Markets

Processed soybeans are the world's largest source of protein for animal feeds and the second largest source of vegetable oil. The use of soybean products in the feed and food industry has increased steadily over the past decades. In December 2014, the world soybean production for the 2014/2015 season was estimated as 312.81 million metric tons, a 9.64% increase over last year's production total of 285.3 million tons (Baize 2014). According to USDA statistics, the United States is the world's largest producer of soybeans at 107.7 million metric tons, followed by Brazil at 94 million metric tons. Argentina, China and India round out the top five soybean producers worldwide.

About 85 percent of the world's soybeans are processed, or "crushed," annually into soybean meal and oil. Approximately 98 percent of the soybean meal that is produced is further processed into animal feed with the balance used to make soy flour and proteins. Of the oil fraction, 95 percent is consumed as edible oil; the rest is used for industrial products such as fatty acids, soaps and biodiesel. Other products made from soy include soy ink widely used in newspaper printing, cosmetics, and industrial dispersing, wetting and emulsifying agents. World compound feed production is fast approaching an estimated 1 billion tons annually.

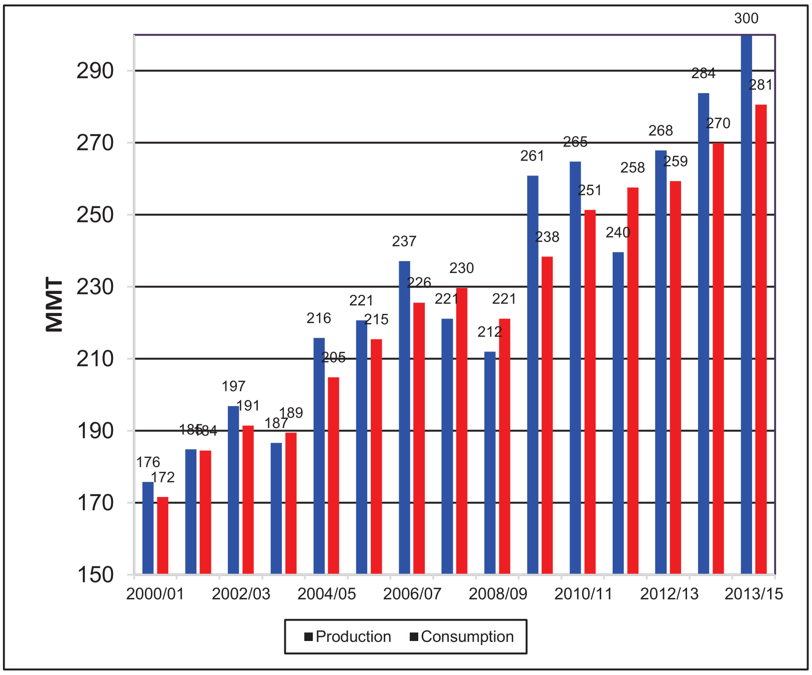


Figure 1. Global Soybean Production and Consumption 2000/01 – 2013/14 and USDA Forecast for 2014/15

Global commercial feed manufacturing generates an estimated annual turnover of over US $370 billion and soybean meals represent the dominant source of protein in animal diets. However, total use and importance of soybeans or soybean products is likely to be higher than indicated by major statistics as a plethora of different soybean products are entering the feed and food chain. This dominant position of soybeans and their products is no doubt associated with their high quality especially with respect to protein and amino acid profile. Figures 1 through 5 show key statistics of soy production (Baize, 2014)

China now is by far the largest importer of soybeans in the world. That is rather amazing considering China was a net exporter of soybeans in the 1995/96 marketing year. Because of its rapid growth in soybean imports China has supplanted the rest of the world as the largest market. In fact, an argument can be made that China's enormous appetite for soybeans has caused the rest of the world to compete for its soybean imports.

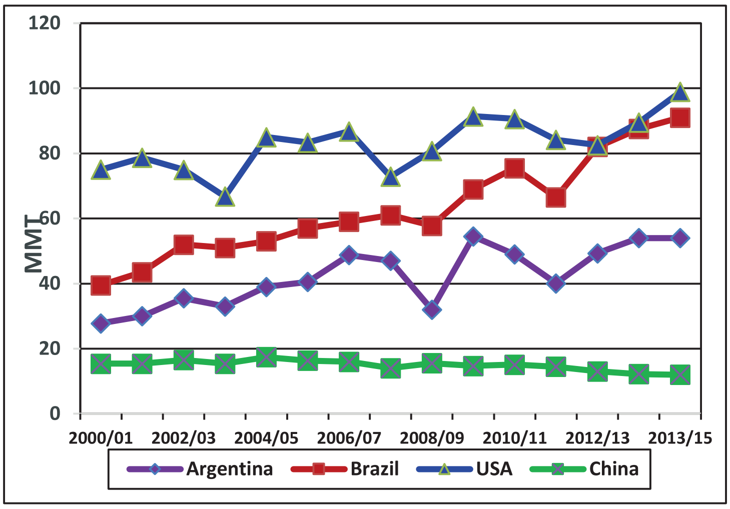


Figure 2. Soybean Production: U.S., Argentina, Brazil and China 2000/01 – 2013/14 and USDA Forecast for 2014/15

In recent years, soybean prices have risen steadily, from $201 per metric ton in 2002, reaching a high in 2012 at $646 per metric ton in 2012. Latest US data put soybean price at an average of $492 per metric ton for 2014. Soybean meal was slightly higher at $524. By contrast, fish meal prices have also tripled since 2002 and have been above $1500 per metric ton since 2012.

As for soybean meal, the current world production is estimated to be in excess of 240 million metric tons which amounts to approximately 69% of all the major protein meals. China is forecast to lead the world with a consumption of more than 56 MMT, followed by the European Union (29 MMT), the United States (almost 27MMT) and Brazil (15 MMT). (USDA/FAS, Oilseed: World Markets and Trade). Globally, demand for soybean meal is also increasing and it is being driven by demand for animal protein as incomes increase in countries like China.

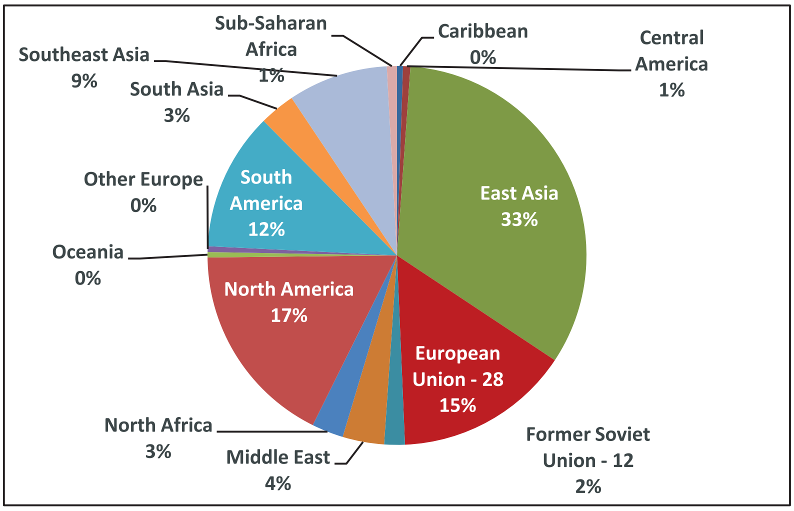


Figure 3. Global Soymeal Consumption by Region for the 2013/14 Marketing Year

So, despite rising prices of soybean products, demand continues to grow due to increased awareness of use of soy as animal feed ingredients and the large variety of uses for human food and other industrial purposes.

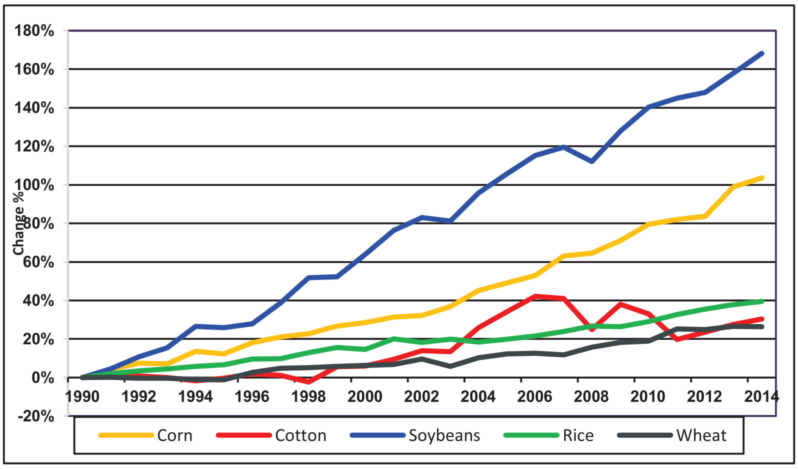


Figure 4. Soybeans, Corn, Wheat, Cotton, and Rice Percentage Change in Global Consumption 1990/91 – 2013/14 and Forecast for 2014/15. Soybean demand is forecast to increase by 168%; corn by 104%, and wheat by only 26% between 1990 and 2014.

As demonstrated in figure 5, by the year 2030, world soybean production is expected to top 350 million metric tons (Matsuda and Goldsmith 2009), and Argentina is forecast to become the world's largest producer. Yields per hectare have doubled since the 1960's, and if further clearing of land for agriculture is to be slowed, technologies and breeding leading to additional increases in yields will be needed.

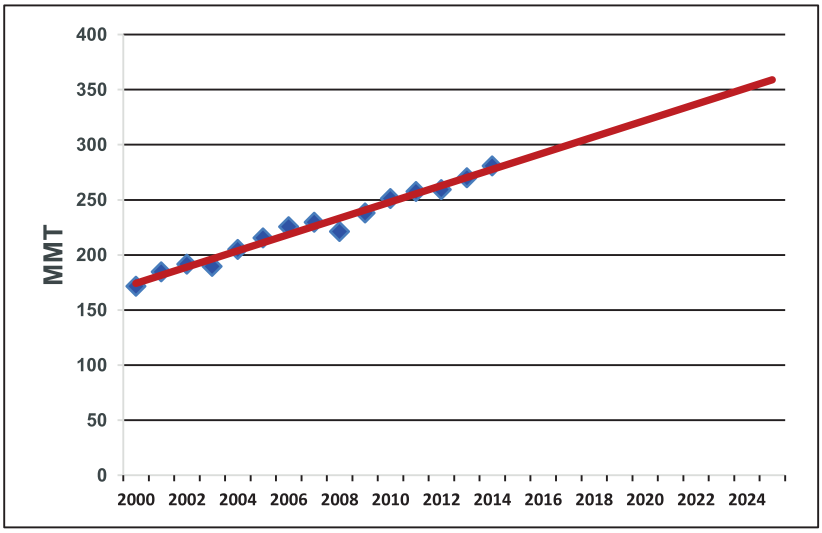


Figure 5. Global soybean consumption 2000/01 – 2014/15 and trend to 2024/25

1.2. Soybean Processing

A large array of different manufacturing processes are applied to obtain the many soy products used in animal and human nutrition. Figure 6 provides a schematic representation of the transformation from soybean into the various products.

The *"crushing"* process of soybeans includes a series of preparatory operations, with crude oil obtained as a major product. The crude oil is refined and separated into lecithin and refined oil used in human as well as animal nutrition; especially in young animal diets. The soybean meals resulting from the oil extraction process, which on a volume basis are the most important products obtained from soybeans, have the de-fatted flakes as an intermediary product that requires further treatment.

1.2.1. Common Processing Methods

The various soybean products are obtained through the separation or extraction of the different components of the soybean. After being cleaned and dehulled, one of three processes is used to separate the soybean oil from the protein meal (this is also called "crushing" or "oil mill" operations). These processes are:

Solvent extraction: This process, which is the one used most commonly around the world, uses hexane to leach or wash (extract) the oil from flaked oilseeds. This method reduces the level of oil in the extracted flakes to one percent or less.

Continuous pressing: This process is performed at elevated temperatures, using a screw press to express the oil from ground and properly conditioned soybeans. The pressed cake is reduced to between 4 percent and 6 percent oil content by this method.

Hydraulic or batch pressing: This is an intermittent pressing operation carried out at elevated temperatures in a mechanical or hydraulic press after the soybeans have been rolled into flakes and properly conditioned by heat treatment. It is the oldest known method of processing oilseeds.

Solvent extraction is the most efficient and widely used process at present where non-polar solvents (commonly hexane and hexane isomers) are used to extract the oil. In the case of solvent extraction, the flakes are de-solventized. All flakes are toasted in order to eliminate the heat-labile anti-nutritional factors. Sometimes the hulls obtained in the preparatory steps are added back to the toasted flakes. This is done in variable degrees resulting in soybean meals with variable levels of fiber and crude protein. When no hulls are added, the higher protein meals are obtained. These are the meals used predominantly in poultry diets and aquaculture diets.

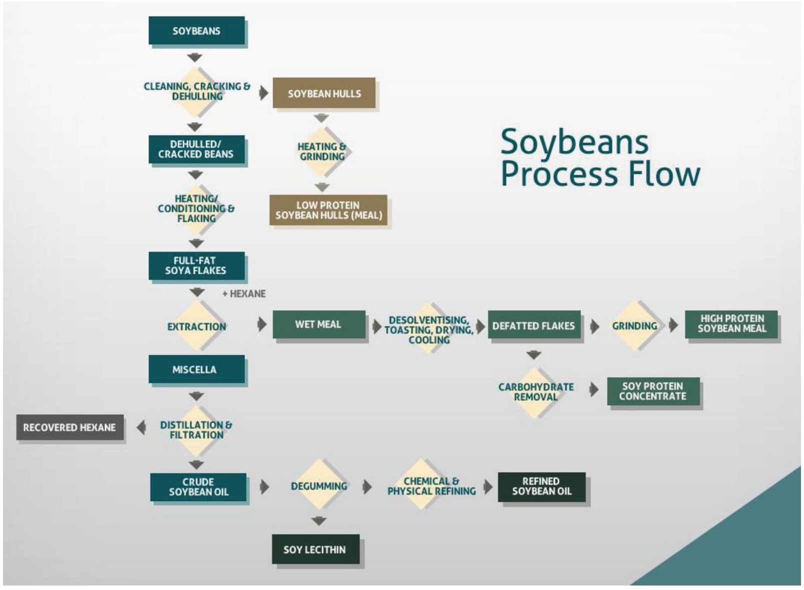


Figure 6. A schematic of the steps in soybean processing
Source: Wilmar International, Singapore

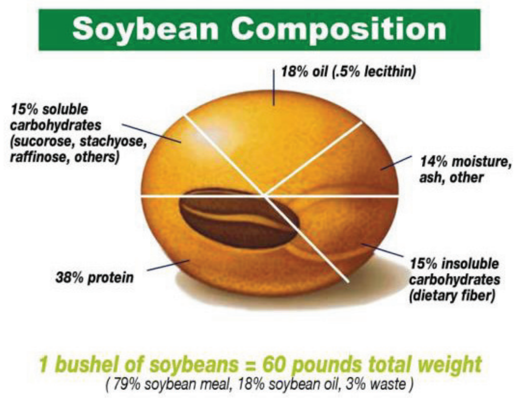


Figure 7. Typical composition of soybeans and conversions that can be used when estimating yields on meal and oil. *Source:* WISHH

Flash de-solvenization or heat vacuum drying of the de-fatted flakes produces the white flakes that are higher in protein quality (solubility) and do not have the undesirable darker color. Through a series of different extraction and precipitation processes, soy protein isolates (SPI) or soy protein concentrates (SPC) are produced. Whereas SPI production is fairly standardized, different methods of extraction are used to obtain the SPC resulting in slightly different compositional characteristics. SPC can be further elaborated (grinding, texturizing, separation on the basis of molecular weight) to obtain a large array of products used in human nutrition. SPI and SPC are used in animal nutrition but are limited to specialty diets due to their relatively high cost. New research initiatives are testing some of the SPC's in the high-protein aquaculture diets used on young fish and carnivorous fish species.

1.2.2 Definition and Application of Soybeans and Soy Products.

The number of soy products currently being used in the feed industry is large, and recent years have seen a dramatic expansion of specialty products based on soybeans. These evolved, value-added products may differ significantly among producers, with each producer applying proprietary knowledge and specialized treatments. Typically, value–added products must be evaluated on the basis of the entity that produces them taking into account the guarantees provided by the manufacturer or distributor. Consistent analysis of these producer-specific products allows classification and the building of an accurate database along with improved confidence about the product. This increased level of knowledge will allow an analysis schedule of decreased intensity and drive increased inclusion rates in diets.

Commodities as well as the value-added products can be classified in a specific class or group of products for which a sufficiently specific description can be developed. For an efficient and correct use - as well as a meaningful interpretation of analytical results - a precise and generally agreed upon definition of the product is needed. Trading, purchasing, formulation, and the entire operation of feed manufacturing depend on the precise referencing of a raw material and the consistent use of the correct name and description. Also the quality control mechanisms that have been introduced in the feed industry require a precise description and classification for all ingredients.

Although many databases and ingredient tables have their own classification system, the most widely recognized system is probably the IFN system (International Feed Name and number), according to Kearl *et al*., (1980) prepared for INFIC, (International Network of feed Information Centers). In this system, ingredients have been divided into eight fairly arbitrary feed classes on the basis of their composition and use (NRC, 1982). The system is widely used in the UK, the US and in Canadian feed composition tables but less so in other countries.

In the IFN system, ingredients are assigned a six digit code with the first digits denoting the International Feed Class Number. With the exception of soybean hay, soybean hulls (class), lecithin, soybean mill run and soybean mill feed (class 4), soy products listed in table 1 fall in the class of protein supplements (5) defined as products that contain more than 20 % crude protein on a dry matter basis. The five digits following the class number is the link between the INF and chemical and biological data in the USA databank (NRC, 1982). The number appears generally on official US ingredient specifications and, although the system may not be used by all feed producers or manufacturers, it provides an easy and systematic reference for quality systems and formulation purposes.

A brief and general description is available for many soy products. This description has the advantage of providing information that is not generally captured in compositional tables. It also provides for a general appreciation of the origin and quality and thus the potential applications or uses in a feed. Although these definitions might differ slightly between different sources, they are in general sufficiently similar to use them interchangeably. The Association of American Feed Control Officials (AAFCO) publishes at regular intervals reference specifications for soybean products (AAFCO, 2006). The definitions listed in table 1 have been used.

Table1. Description and Classification of Soybean Products most often used in animal feeds, in alphabetical order. Adapted from AAFCO Official Publication 2006 and Canadian Food Inspection Agency, 2003. The products listed in bold print are used in aquaculture feeds.

Full Fat Soybeans, Extruded (AAFCO: Ground Extruded Whole Soybeans) is the meal product resulting from extrusion by friction heat and/or steam of whole soybeans without removing any of the component parts. It must be sold according to its crude protein, fat and fiber content. IFN 5-14-005.

1. **Full Fat Soybeans, Raw (AAFCO:** Ground Soybeans) are obtained by grinding whole soybeans without cooking or removing any of the oil. IFN 5-04 -596.
2. **Full Fat Soybeans, Roasted (AAFCO:** Heat Processed Soybeans) is the product resulting from heating whole soybeans without removing any of the component parts. It may be ground, pelleted, flaked or powdered. It must be sold according to its crude protein content. Maybe required to be labeled with guarantees for maximum crude fat, maximum crude fiber and maximum moisture (CFIA 2003). IFN 5-04-597.

3. **Kibbled Soybean Meal** is the product obtained by cooking ground solvent extracted soybean meal, under pressure and extruding from an expeller or other mechanical pressure device. It must be designated and sold according to its protein content and shall contain no more than 7% crude fiber. IFN 5-09-343.

4. **Soy Flour** is the finely powdered material resulting from the screened and graded product after removal of most of the oil from selected sound cleaned and dehulled soybeans by a mechanical or solvent extraction process. It must contain no more than 4.0% crude fibers. Some organizations also require labeling guarantees for minimum crude protein and maximum crude fat and moisture. IFN 5-12-177.

5. **Soy Grits** is the granular material resulting from the screened and graded product after removal of most of the oil from selected, sound, clean and dehulled soybeans by a mechanical or solvent extraction process. It must contain no more than 4% crude fiber. Soybean grits mechanical extracted: IFN 5-12-176. Soybean grits solvent extracted: IFN 5-04-592.

6. **Soy Lecithin** or Soy Phosphate is the mixed phosphatide product obtained from soybean oil by a degumming process. It contains lecithin, cephalin and inositol phosphatides, together with glycerides of soybean oil and traces of tocopherols, glucosides and pigments. It must be designated and sold according to conventional descriptive grades with respect to consistence and bleaching. IFN 4-04-562.

7. **Soy Protein Concentrate** is prepared from high quality, sound, dehulled soybean seeds by removing most of the oil and water soluble non-protein constituents from selected, sound, cleaned, dehulled soybeans (CFIA 2003) and must contain not less than 65% protein on a moisture-free basis. It shall be labeled with guarantees for minimum crude

protein, maximum crude fat, maximum crude fiber, maximum ash and maximum moisture. IFN 5-32-183.

8. **Soy Protein Isolate** is the major proteinaceous fraction of soybeans prepared from dehulled soybeans by removing the majority of non-protein components, and contains not less than 90% protein on a moisture-free basis. The CFIA (2003) adds that the original material must consist of selected, sound, cleaned, dehulled soybeans and that it shall be labeled with guarantees for minimum crude protein (90%), maximum ash and maximum moisture. IFN Number 5-08-038 (CFIA lists this product with the IFN Number 5-24-811.

9. **Soybean Flour Solvent Extracted (or Soy flour)** is the finely powdered material resulting from the screened and graded product after removal of most of the oil from dehulled soybeans by a solvent extraction process. It shall contain less than 4 percent crude fiber. It shall be labeled with guarantees for minimum crude protein, maximum crude fat, maximum crude fiber and maximum moisture. IFN 5-04-593.

10. **Soybean Hulls** consist primarily of the outer covering of the soybean. IFN-1-04-560.

11. **Soybean Meal, Mechanical Extracted** is the product obtained by grinding the cake or chips which remain after removal of most of the oil from soybeans by a mechanical extraction process. It must contain no more than 7% crude fibers. It may contain an inert, non-toxic conditioning agent either nutritive or non-nutritive or any combination thereof, to reduce caking and improve flowability in an amount not to exceed that necessary to accomplish its intended effect and in no-case exceed 0.5%. The name of the conditioning agent must be shown as an added ingredient. IFN 5-04-600.

12. **Soybean Meal, Dehulled, Solvent-Extracted** is obtained by grinding the flakes remaining after removal of most of the oil

from dehulled soybeans by a solvent extraction process. It must contain no more than 3.3% crude fibers. It may contain an inert non-toxic conditioning agent either nutritive or non-nutritive or any combination thereof, to reduce caking and improve flowability in an amount not to exceed that necessary to accomplish its intended effect and in no-case to exceed 0.5%. The name of the conditioning agent must be shown as an added ingredient. IFN 5-04-612. It may also be required to be labeled with guarantees for minimum crude protein, maximum crude fat and maximum moisture (CFIA 2003).

13. **Soybean Meal, Solvent-Extracted,** is the product obtained by grinding the flakes which remain after removal of most of the oil from soybeans by a solvent extraction process. It must contain no more than 7% crude fibers. It may contain an inert, non-toxic conditioning agent either nutritive or non-nutritive and any combination thereof, to reduce caking and improve flowability in an amount not to exceed that necessary to accomplish its intended effect and in no-case exceed 0.5%. It shall contain less than 7 percent crude fiber. The CFIA (2003) specifies that it shall be labeled with guarantees for minimum crude protein, maximum crude fat and maximum moisture. IFN 5-04-604.

14. **Soybean Oil consists** of the oil from soybean seeds that are commonly processed for edible purposes. It consists predominantly of glyceride esters of fatty acids. If an antioxidant(s) is used, the common name or names shall be indicated on the label. It shall be labeled with guarantees for maximum moisture, maximum insoluble matter, maximum un- saponifiable matter and maximum free fatty acids. IFN 4-07-983.

The list in Table 1 demonstrates the large diversity of soy products and different methods of producing them. Although it only represents the major soy products produced, it provides a brief description of how the product is obtained and for some products a compositional reference point. The common name and IFN is provided which allows for a consistent and non-equivocal use of ingredients, important in quality systems. The description gives an adequate back ground of the products for trading and classification purposes, references in quality systems and production purposes. It is sufficiently precise to provide clear reference points for product definition and contract agreements but general enough to cover a substantial variation in composition and production processes. For proper use of an ingredient, additional analytical data should complement the information provided in the description. However, for analytical purposes the descriptions provide general back ground information as to what can be expected and how analysis should be carried out or what results may be expected. When considering formulation objectives, the description only serves as a classification aide and more precise compositional data will be necessary.

As previously mentioned, the products listed in Table 1 only represent the major soy products produced. At present, a large number of additional specialty products are marketed and the list does not adequately reflect the acceleration seen in the development of new soy products mostly branded products.

The most important products in terms of volume of use are soybean meals (SBM), solvent extracted, or dehulled (items 13 and 14, Table 1) resulting from the original use of soybeans i.e. the removal of oil. This is also the case for the mechanical extracted SBM (item 12) although this type of SBM is much less common. Full fat soybeans (FFSB; items 1,2,3) in raw, extruded or roasted form are defined and their use (particularly for the extruded and roasted forms) is increasing due to their high energy content, especially in formulations where animal products (meat and bone meals and fats) were previously of interest. One fiber-rich product is listed as soybean hulls (item 11). The interest in soybean hulls is important and an increasing application in the compound feed industry, especially for ruminants. It is rarely used in aquaculture diets, except for feeds formulated for the herbivorous grass carp (*Ctenopharyngodon idella*), popular in China.

Soy flour and soy grits are primarily products destined for human consumption although minor amounts may find an application in specialty animal diets. Technological modifications of these products have produced different types of flour and grits. They are further classified and commercialized according to their application objectives with the main differences being the level of fat content or heat treatment.

Soy lecithin is produced when the oil fraction is “de-gummed” and it has several human and animal feed uses. The three main phosphatides in commercial soy lecithin are phosphatidyl choline (also called "pure" or "chemical" lecithin to distinguish it from the natural mixture), phosphatidyl ethanolamine (popularly called "cephalin"), and phosphatidyl inositol (also called inositol phosphatides). Commercial soy lecithin also typically contains roughly 30%-35% unrefined soy oil. Because it’s readily available from plentiful soybean crops all over the world, it’s the cheapest and easiest type of lecithin to mass manufacture. Lecithin is used as an emulsifier in many types of human food products and animal feeds and is often added to fish feed formulations in small doses.

With the increased complexity of production processes aimed at removing ANF and improving protein digestibility, a clear understanding of the products and the production process becomes more important. Quality difference between producer/suppliers for these products can be substantial, especially for the more evolved products. These differences need to be verified and understood at the feed manufacturer’s level. It remains the responsibility of the user to carry out the needed quality analysis and classify suppliers and products accordingly. Reliable manufacturer's information is, of course, important but verification remains the basis of this tool and of the overall quality assurance program. The quality of the information provided by the manufacturer must be an integral part of the "supplier classification process".

The quality of ingredients plays a determining role in the level at which these ingredients are used in animal diets. Quality criteria used to determine the inclusion level for an ingredient go beyond the standard nutrient levels, and have often more to do with residual ANF, storage and contamination and the physiological characteristics of the animal. The inherent variation in quality and chemical characteristics associated with these ingredients make repeated quality analyses necessary which in turn will determine more precisely the inclusion levels employed. The nutritionist's experience and interpretation of the quality analyses play a major role in defining the final inclusion level used in particular diets. Table 2 gives estimates of maximum inclusion levels of each product under practical conditions of diet formulation. The inclusion levels suggested are for inclusion in complete diets and are thus necessarily general. The levels need to be further defined for each feed manufacturer, the manufacturing process and the feed being formulated. Some of the maxima suggested are not defined by any inability of the animal to use the nutrients in a given product, but rather by the effects of specific nutrients on carcass or product quality. Such is the case for extruded or roasted FFSB or soybean oil. Other maxima are controlled by economic considerations. While higher inclusions in diets may be possible, the added cost of increasing those levels needs to be evaluated.

Table 2. Application of soybean products as presented in the 2nd Edition of Manual of soy quality analyses by U.S. Soybean Export Council (ASA-IM)

PRODUCT	SPECIES					LEVEL %
	Poultry	Swine	Ruminant	Aqua	Pet	
Condensed Soybean			X			10
Dried Soybean			X			15
Ground Extr. Whole	X	X	X	X	X	3[5]
Ground Soybean Hay		X	X			20
Ground Soybeans		X	X			15
Heat Processed	X	X	X			15
Kibbled Soybean	X	X	X	X	X	10 (Y)
Soy Lecithin Or Soy	X	X	X	X	X	3
Soy Protein	X	X	X	X	X	7 (Y)
Soy Protein Isolate	X	X	X	X	X	10 (Y)
Soybean Feed, Solv.	X	X	X	X	X	5 (Y)
Soybean Flour	X	X	X			40
Soybean Hulls	X	X	X			25
SBM Mech Extract	X	X	X			30
SBM Dehulled Solv.	X	X	X	X	X	35
SBM Solv. Extract	X	X	X	X		35
Soybean Mill Feed	X	X	X	X		10
Soybean Mill Run	X	X	X	X		10
Soybean Oil	X	X	X	X	X	8

[1]Suggested upper-use levels in diets of different domestic species; this will vary with age of animal, quality, composition and analysis of product; does not include young animal diets unless specifically indicated. Detailed and extensive analyses will allow discretionary changes in usage level.

[2]Species: Production diets (growing/finishing) for Poultry, Swine, Ruminants, Aqua (salmonids); Pets (dogs).

[3]On a diet dry matter basis. "Y" indicates primarily in young animal diets.

[4]Higher levels may be used in salmon and trout grower, finisher diets.

[5]Maximum inclusion of oil in Ruminant diets should not exceed 2%.

1.3. Chemical and Nutritional Composition of Soybean Products.

The compositional data provided in the Tables 3 and 4 are better descriptors of the nutritional characteristics of soybean products.

The table values provide means based on a large number of samples covering many years and a wide range in origin. They cannot be used as standard values but only as reference points around which analysis of individual samples should be situated if they are to be identified by the specific ingredient name.

For most users of soy products the detailed nutrient concentrations serve as a basis to formulate diets and to calculate total nutrient supply to animals. However, for precise formulations the analytical data on the ingredient in the plant should be used. The use of the table values, especially because of the large contribution that soy products make to the protein and amino acid supply, may lead to significant variations in nutrients between the formulated value and the real diets.

The compositional data in Table 4 includes nutrients and ANF that can be directly analyzed in a large and well equipped laboratory. Routine analyses, as carried out in standard quality control procedures or smaller laboratories, mainly concern the proximate analysis, the Van Soest fiber components (with the exception of lignin) and the minerals calcium and phosphorus. These analyses (especially the proximate) are most often used to derive other nutrient values such as amino acids or energy. In advanced formulation systems they are generally combined with estimates of digestibility for each individual nutrient. No digestibility data are included here as this information is not necessarily the result of direct observations but rather of literature compilations and research conducted by feed compounders. Thus digestibility data used in formulation systems can differ considerably among users and are generally considered proprietary information.

Table 3. Composition of major soy protein ingredients used in animal feeds.[1,2,3]

	Unit	Full fat soybeans, roasted	SBM mechanical extracted	SBM solvent extracted 44	SBM solvent Extracted 48	SBM solvent extracted 50	Soybean hulls	Soy protein concentrate	Soy protein isolate
International feed number		5-04-597	5-04-600	5-04-600	5-04-604	5-04-604	1-04-560	5-32-183	5-24-811
Dry matter	%	89.44	89.80	88.08	87.58	88.20	89.76	91.83	93.38
Crude protein	%	37.08	43.92	44.02	46.45	48.79	12.04	68.60	85.88
Crude fiber	%	5.12	5.50	6.26	5.40	3.42	34.15	1.65	1.32
Ether extracts	%	18.38	5.74	1.79	2.13	1.30	2.16	2.00	0.62
Ash	%	4.86	5.74	6.34	6.02	5.78	4.53	5.15	3.41
NDF[4]	%	12.98	21.35	13.05	11.79	9.95	56.91	13.50	-
ADF[4]	%	7.22	10.20	8.76	7.05	5.00	42.05	5.38	-
ADL[4]	%	4.30	1.17	0.75	0.90	0.40	2.05	0.40	-
Starch	%	4.66	7.00	5.51	5.46	3.28	5.95	-	-
Total sugars	%	6.70	9.76	9.06	9.17	9.29	1.40	-	-
Gross energy	kcal/kg	5013	-	4165	4130	4120	3890	4280	5370
Lysine	%	2.34	3.50	2.85	2.89	3.00	0.73	4.59	5.26
Threonine	%	1.53	2.21	1.80	1.84	1.90	0.73	2.82	3.17
Methionine	%	0.52	0.80	0.62	0.63	0.67	0.14	0.87	1.01
Cystine	%	0.55	0.77	0.68	0.73	0.73	0.16	0.89	1.19
Tryptophane	%	0.49	0.74	0.56	0.63	0.65	0.12	0.81	1.08
Calcium	g/kg	2.62	2.96	3.12	3.07	2.68	4.96	2.37	1.50
Phosphorus	g/kg	5.70	6.64	6.37	6.37	6.36	1.59	7.63	6.50
Magnesium	g/kg	2.80	2.84	2.72	3.03	2.88	2.23	1.85	0.80
Potassium	g/kg	15.93	20.28	19.85	22.00	20.84	12.15	12.35	2.75
Sodium	g/kg	0.29	0.33	0.18	0.18	0.88	0.10	0.55	2.85
Linoleic acid – C18:2	%	9.70	2.87	0.64	0.80	0.56	1.21	-	-

[1]All values as fed basis

[2]SBM=soybean meal.

[3]Source: compilation of NRC, INRA-AFZ, CVB, FEDNA and selected supplier

[4]NDF = neutral detergent fiber; ADF = acid detergent fiber; ADL = acid detergent lignin (klason lignin)

Source: Van Eys, Manual of quality analyses for soybean products in the feed industry, 2nd ed

Table 4. Analytical characteristics of common types of soy protein products. [1,2]

Product type	Unit	Soybean seeds, raw	SBM	Enzyme treated SPC	Alcohol extracted SPC	SPI
Moisture	%	10 – 12	10 – 12	6 – 7	6 – 7	6 – 7
Crude protein	%	33 – 37	42 – 50	55 – 60	67 – 70	>85
Fat	%	17 - 20	0.9 – 3.5	2.5	0.5 – 3.0	0.1 – 1.5
Ash	%	4.5 – 5.5	4.5 – 6.5	6.2 – 6.8	4.8 – 6.0	2.0 – 3.5
Oligosacharides	%	14	15	<1.0	<3.5	<0.4
Stachyose	%	4 – 4.5	4.5 – 5	<0.3	1 - 3	<0.2
Raffinose	%	0.8 – 1	1 – 1.5	<0.2	<0.2	<0.1
Verbascose	%	-	0.3 – 0.4	-	-	-
Trypsin inhibitors	mg/g	25 – 50	1.6 – 5.0	1 – 2	2 – 3	<1
Glysinin	mg/g	150 – 200	20 – 70	<0.01	<0.1	<0.01
B-conglycinin	mg/g	50 – 100	3 – 40	<0.01	<0.01	<0.005
Lectins	ppm	2100 – 3500	20 – 600	<1.0	<1.0	<1.0
Saponins	%	0.5	0.6	0	0	0
Phytic acid bound	%	0.38	0.42 – 0.49	0.6	0.6	-

Note. SBM = defatted soybean meal; SPC = soy protein concentrate; SPI = soy protein isolate

[1]Adapted from: Hansen (2003), Peisker (2001), Fasina et al (2004), Maenz et al (1999), CVB (1999), Ruiz (2011).

[2]All values as fed basis

Source: Compilation by Van Eys, Manual of quality analyses for soybean products in the feed industry, 2nd ed.

While a large number of compositional tables and publications for soybean products exist, those data cannot be considered as standard values, especially not for trading purposes. For trading and contractual purposes they are too detailed and thus unpractical. Furthermore, they do not provide the required borderline minimum or maximum values for limited number readily identifiable parameters.

A limited number of official standards have been published to start with the basic material: whole, untreated soybeans or seeds (IFN 5-04-610). As is the case for all other grains and seeds the USDA publishes official standards for soybean grains as defined under the United States Grain Standards Act. These standards do not generally change much over time and under the act soybeans are defined as grains that consists of 50 percent or more of whole or broken soybeans (Glycine max (L) Merr.) that will not pass through an 8/64''round hole sieve (3183 microns) and does not contain more than 10.0 percent of other grains for which standards have been established under the United States Grain Standards Act (USDA, 2007).

For trading purposes – especially in view of specific applications and export requirements – additional specifications are provided by dividing soybeans into classes and grades. Only two classes of soybeans have been defined (yellow soybeans and mixed soybeans) but 5 grades are specified. The grades and grade requirements for the major export countries (USA, Brazil and Argentina) are similar. However, while Brazil and Argentina have a special export grade, the United States does not define a specific export grade as soybeans are exported from the US at any pre-defined specification or grade. The USDA (2007) description of grades is provided in Table 5.

Next to whole soybeans, only three soybean products (two soybean meals and soybean oil) have standard values. Used as official reference standards, they have been developed by the National Oil Processors Association (NOPA, 1999, 2011) and are also published by the American Soybean Association (ASA, 2005) in the Soy Importers Guide. These standards are now widely accepted and provide minimums or maximums on only a few, easily identifiable, key parameters. In the case of soybean meals, their main purpose is the classification of the soybean meal products into two main categories: solvent extracted SBM and de-hulled, hi-pro, SBM.

For soybean oil, the NOPA standards refer to crude de-gummed soy bean oil mainly with food application purposes in mind. These standards serve as a general guide for transactions, thus assuring a minimal degree of quality and consistency in at least the three main types of soy products being traded. However, the standards and trading guidelines proposed by NOPA are not binding.

Table 5. U.S. grades and grade requirements for soybeans.

Grading factors	**Grades U.S. Nos**			
	1	2	3	4
Damaged kernels: - Heat (part of total) - Total	 0.2 2.0	 0.5 3.0	 1.0 5.0	 3.0 8.0
Foreign material	1.0	2.0	3.0	5.0
Splits	10.0	20.0	30.0	40.0
Soybeans of other colour[1]	1.0	2.0	5.0	10.0

	Maximum count limits of			
Other materials: - Animal filth	9	9	9	9
Castor beans	1	1	1	1
Crotalaria seeds	2	2	2	2
Glass	0	0	0	0
Stones[2]	3	3	3	3
Unknown foreign substance	3	3	3	3
Total[3]	10	10	10	10

U.S. sample grades are soybean that:

a) Do not meet the requirements for U.S. Nos 1,2,3, or 4; or
b) Have a musty, sour, or commercially objectionable odor (except garlic odor); or
c) Are heating or otherwise of distinct lower quality.

[1]Disregard for mixed soybeans

[2]In addition to the maximum count limit, stones must exceed 0.1 percent of the sample weight

[3]Includes any combination of animal filth, castor beans, crotalaria seeds, glass, stones, and unknown foreign substances. The weight of stones is not applicable for total other material. (USDA, 2007).

These standards principally serve the trading and marketing of US soybean products within the USA but due to their wide acceptance, their impact goes well beyond US meals (and oils) as they are generally applied to compare and benchmark soybean products from other origins.

Solvent extracted soybean meal can be the result of blending back soybean hulls in the dehulled meal. The blending of different types of soybean meals or soybean components at the point of shipping is allowed under NOPA regulations and standards for minimum blending procedures are provided. As a matter of fact, this can be the source of a significant variation in quality and chemical composition. However, blending of soybeans is not permitted. For soybean meals, only soy hulls, soybean mill run and soybean mill feed are permitted to be blended with soybean meals before the point of sampling. The blending must lead to a meal of uniform quality representative of the contract terms.

Dehulled soybean meal is of particular interest to fish feed producers because most fish feed formulators seek to keep fiber as low as possible. One of the challenges with using plant protein is the typically high fiber associated with these ingredients.

Table 6. Specifications for solvent extracted and dehulled soybean meal (%).

	Min/Max	Solvent extracted SBM	Dehulled SBM
Moisture	Max	12	12
Protein	Min	44	47.5 – 49.0
Fat	Min	0.5	0.5
Crude fiber	Max	7	3.3 – 3.5
Anti-caking agent	Max	0.5	0.5

Source : (National Oilseed Processor Association (NOPA), 2011)

The National Oil Processor Association (NOPA) in the U.S. sets quality standards and describes the various processed products derived from soy. The NOPA quality standards applied to domestic shipments are also followed for international shipments although contracts may be patterned after North American Export Grain Association (NAEGA) or the Grain and Feed Trade Association (GAFTA). This ensures quality and customer satisfaction.

For SBM, the NOPA standards clearly aim at providing a minimum number of primary quality characteristics and as such are only a basis for contract specifications (Table 6). Meals purchased under NOPA contract specifications will therefore still need additional analysis. In order to provide greater quality assurances and meet the nutritional requirements of the feed compounder or nutritionist, additional recommendations have been added by NOPA (Table 7).

Table 7. Recommended additional specifications for soybean meal.

Ash	<7.5%
Acid insoluble ash (silica)	<1%
Total lysine	>2.85% (basis 88% dry matter)
Digestible lysine	Equal or >88% of total lysine
Protein solubility in 0.2% KOH	78-85% (or more if urease is within specifications
Protein dispersibility index	15 – 40%
Urease activity	0.02 – 0.30 pH unit rise
Trypsin inhibitors	< 4 mg/g of meal
Bulk density	57 – 64 g / 100 cc
Screen analysis (mesh)	95% thru #10 mesh, 45% thru #20 mesh, 6% thru #80 mesh
Texture	Uniform, free flowing, no lumps, cakes, dust
Color	Uniform particle colors of light tan to light brown
Odor	Fresh, not musty, not sour, not like ammonia, not burned
Contaminants	Free of urea, free of ammonia, free of Mycotoxins and molds, free of pesticides, grains, and seeds

These are only recommendations that apply in a non-binding manner to all soybean meals. Rather than guidelines, they should be regarded as further suggestions to both producers of soybean meal and buyers, provided in an effort to improve the quality of US soybean meals. Under practical conditions there remains a large variation around these recommendations and from a feed compounder's point of view, information on quality requirements for SBM needs to be still more detailed. Also, new parameters have been added and more recently evaluations have changed slightly. For instance there is a definite tendency for KOH values to shift to the high end of the established range (close to the 85 % value).

Soybean meal has one of the best amino acid profiles relative to other vegetable –protein meals. Relative to fishmeal, soybean meal is rich in lysine, but deficient in methionine and cysteine. Utilization of soybean meal by fish is variable, but with adequate oil, mineral, and possible amino acid supplementation, properly toasted soybean meal is capable of replacing much of, if not all, the fish meal in diets for many aquaculture species. Soybean meal is readily available in most world markets at 25 to 50% of the cost of fish meal, but it contains 66 to 75% of the crude protein found in fishmeal. In general, soybean meal is much more uniform and standard, compared to fishmeal. Soybean meal is traded on the basis of weight, moisture, protein, fat and urease level.

References

Please find all references from Chapter VI A at the end of Chapter VI B.

CHAPTER – VI A

THE SOYBEAN AND ITS PRODUCTS

Author
R.S.N. Janjua
Country Representative,
American Soybean Association
World Initiative for Soy in Human Health
Karachi – Pakistan
E-mail: janjua@soypak.org

Editor
Karen L. Veverica
Director, E.W. Shell Fisheries Center
School of Fisheries, Aquaculture and Aquatic Sciences
Auburn University, AL USA

1. INTRODUCTION TO SOY AND SOY PRODUCTS

Soybeans (US) or Soya bean (UK) is a species of legume that originated in China and has been used in East Asia for more than three millennia. More recently, it has been has been successfully cultivated around the world. The plant is classed as an oilseed rather than a pulse by the UN Food and Agriculture Organization. Soybean is a highly versatile crop and has many uses but it has become a major source of protein-rich feed ingredient for diets used in the poultry, swine and aquaculture industries throughout the world.

The soybean plant [Glycine max (L.) Merrill], is bushy with height ranging from 0.75 – 1.25 m that branches sparsely and densely depending on cultivators and growing conditions. The pods, stems and trifoliate leaves (sometimes with 5 leaflets) are covered with fine brown or gray pubescence. Soybean crops can build soil fertility by fixing large amount of atmospheric nitrogen using bacteria located in their root nodules.

There are over 2,500 varieties of soybeans that vary greatly in size, shape and color. Shapes vary from flat to spherical and colors range from yellow to green, brown and black. Modern varieties, mainly grown for their oil content, are generally spherical in shape with yellow or green as the accepted seed coats.

The soybean consists of two cotyledons which represent approximately 90% of the weight, a seed coat or hull (8% of weight), and two much smaller and lighter structures the hypocotyls and the plumula. The cotyledons contain the proteins and lipids (oils) that constitute the main nutritional components of the soybean products obtained from soybeans. They are also the main storage area for the carbohydrates and various other components of importance, most notably the enzymes (lipoxygenase, urease) and the anti-nutritional factors (ANF).

Advances in soy processing have allowed for the increased use of soy in both human and animal nutrition following proper treatment or extraction of the soybeans to eliminate many of the anti-nutritional factors (ANF). Soy is one of the few plants that provide a complete protein as it contains all eight amino acids essential for human health. The digestibility of the protein fraction is high and the amino acid profile, when matched with a grain provides a close match to animal requirements. For this reason, and because of the higher amount of protein produced by soybeans per unit area of land compared to any other crop, it has been called the **“wonder bean”** and **“nature’s miracle bean”.**

1.1. Production, Consumption and Markets

Processed soybeans are the world's largest source of protein for animal feeds and the second largest source of vegetable oil. The use of soybean products in the feed and food industry has increased steadily over the past decades. In December 2014, the world soybean production for the 2014/2015 season was estimated as 312.81 million metric tons, a 9.64% increase over last year's production total of 285.3 million tons (Baize 2014). According to USDA statistics, the United States is the world's largest producer of soybeans at 107.7 million metric tons, followed by Brazil at 94 million metric tons. Argentina, China and India round out the top five soybean producers worldwide.

About 85 percent of the world's soybeans are processed, or "crushed," annually into soybean meal and oil. Approximately 98 percent of the soybean meal that is produced is further processed into animal feed with the balance used to make soy flour and proteins. Of the oil fraction, 95 percent is consumed as edible oil; the rest is used for industrial products such as fatty acids, soaps and biodiesel. Other products made from soy include soy ink widely used in newspaper printing, cosmetics, and industrial dispersing, wetting and emulsifying agents. World compound feed production is fast approaching an estimated 1 billion tons annually.

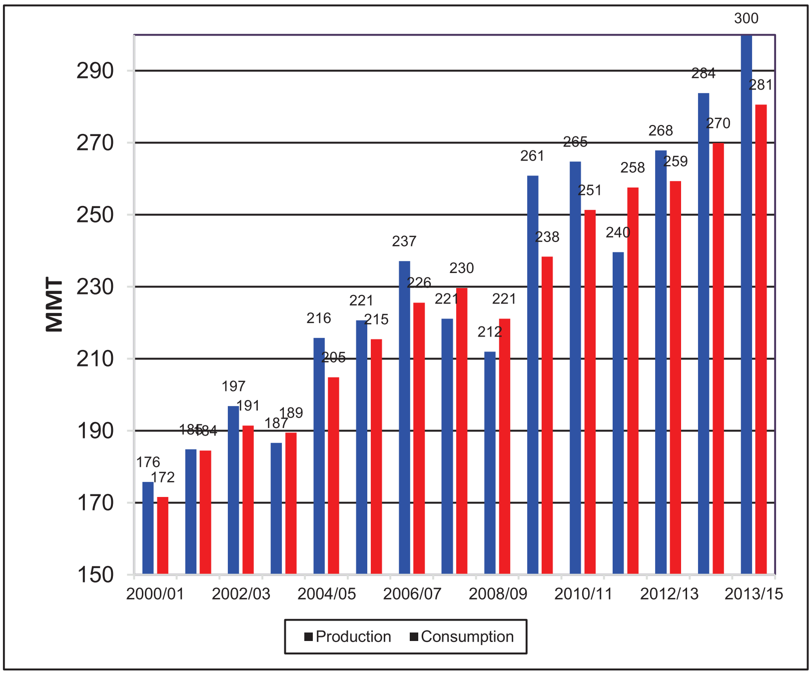


Figure 1. Global Soybean Production and Consumption 2000/01 – 2013/14 and USDA Forecast for 2014/15

Global commercial feed manufacturing generates an estimated annual turnover of over US $370 billion and soybean meals represent the dominant source of protein in animal diets. However, total use and importance of soybeans or soybean products is likely to be higher than indicated by major statistics as a plethora of different soybean products are entering the feed and food chain. This dominant position of soybeans and their products is no doubt associated with their high quality especially with respect to protein and amino acid profile. Figures 1 through 5 show key statistics of soy production (Baize, 2014)

China now is by far the largest importer of soybeans in the world. That is rather amazing considering China was a net exporter of soybeans in the 1995/96 marketing year. Because of its rapid growth in soybean imports China has supplanted the rest of the world as the largest market. In fact, an argument can be made that China's enormous appetite for soybeans has caused the rest of the world to compete for its soybean imports.

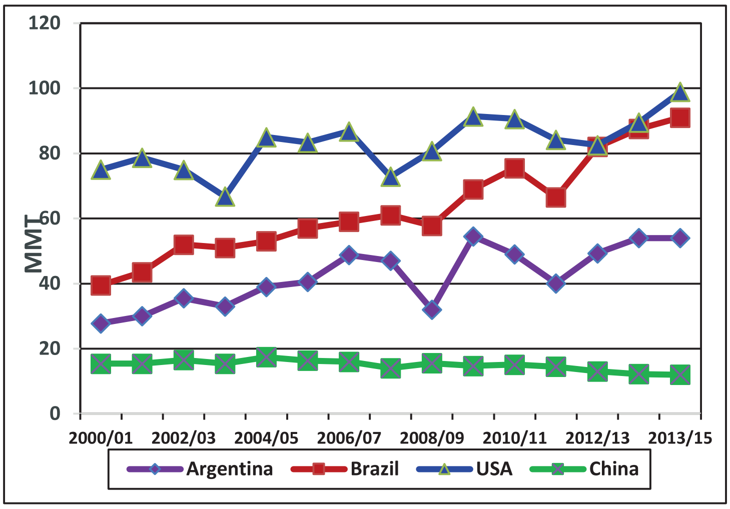


Figure 2. Soybean Production: U.S., Argentina, Brazil and China 2000/01 – 2013/14 and USDA Forecast for 2014/15

In recent years, soybean prices have risen steadily, from \$201 per metric ton in 2002, reaching a high in 2012 at \$646 per metric ton in 2012. Latest US data put soybean price at an average of \$492 per metric ton for 2014. Soybean meal was slightly higher at \$524. By contrast, fish meal prices have also tripled since 2002 and have been above \$1500 per metric ton since 2012.

As for soybean meal, the current world production is estimated to be in excess of 240 million metric tons which amounts to approximately 69% of all the major protein meals. China is forecast to lead the world with a consumption of more than 56 MMT, followed by the European Union (29 MMT), the United States (almost 27MMT) and Brazil (15 MMT). (USDA/FAS, Oilseed: World Markets and Trade). Globally, demand for soybean meal is also increasing and it is being driven by demand for animal protein as incomes increase in countries like China.

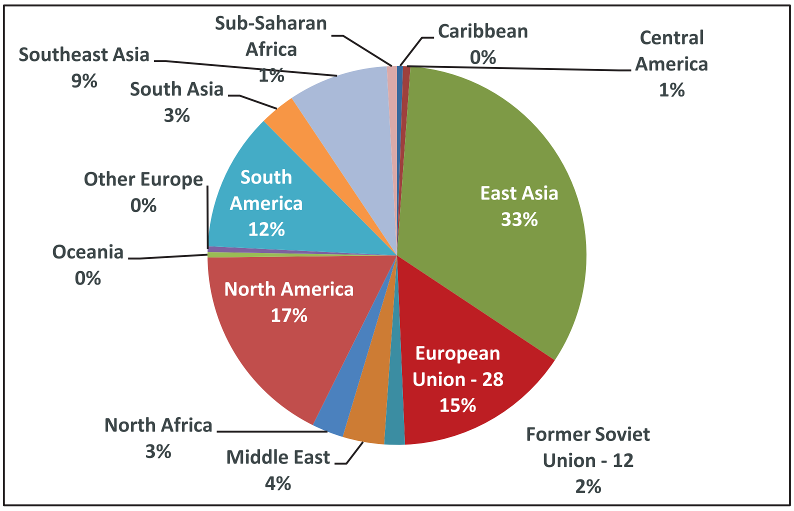


Figure 3. Global Soymeal Consumption by Region for the 2013/14 Marketing Year

So, despite rising prices of soybean products, demand continues to grow due to increased awareness of use of soy as animal feed ingredients and the large variety of uses for human food and other industrial purposes.

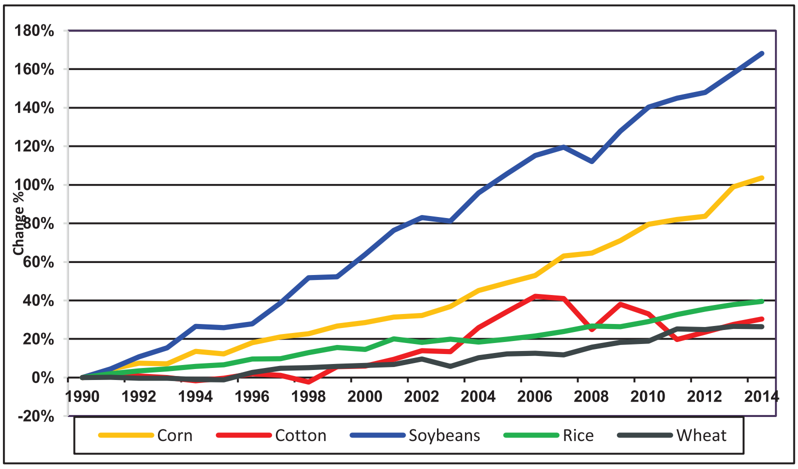


Figure 4. Soybeans, Corn, Wheat, Cotton, and Rice Percentage Change in Global Consumption 1990/91 – 2013/14 and Forecast for 2014/15. Soybean demand is forecast to increase by 168%; corn by 104%, and wheat by only 26% between 1990 and 2014.

As demonstrated in figure 5, by the year 2030, world soybean production is expected to top 350 million metric tons (Matsuda and Goldsmith 2009), and Argentina is forecast to become the world's largest producer. Yields per hectare have doubled since the 1960's, and if further clearing of land for agriculture is to be slowed, technologies and breeding leading to additional increases in yields will be needed.

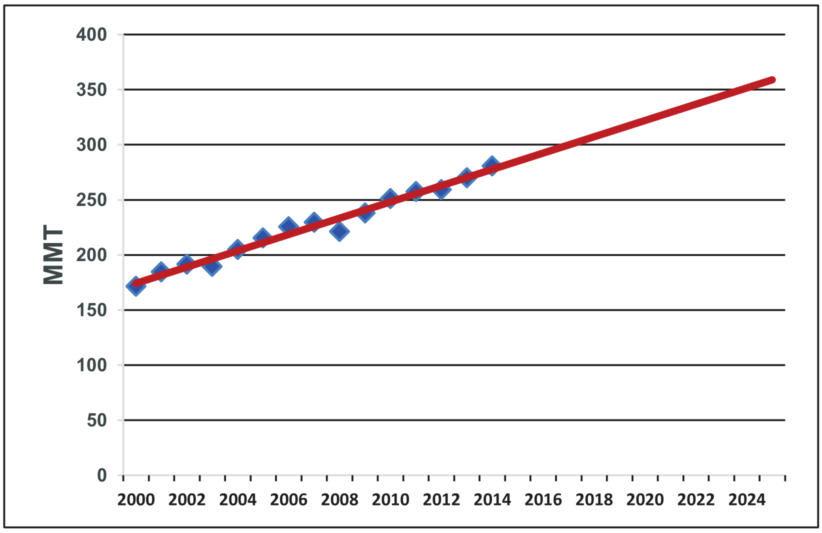


Figure 5. Global soybean consumption 2000/01 – 2014/15 and trend to 2024/25

1.2. Soybean Processing

A large array of different manufacturing processes are applied to obtain the many soy products used in animal and human nutrition. Figure 6 provides a schematic representation of the transformation from soybean into the various products.

The *"crushing"* process of soybeans includes a series of preparatory operations, with crude oil obtained as a major product. The crude oil is refined and separated into lecithin and refined oil used in human as well as animal nutrition; especially in young animal diets. The soybean meals resulting from the oil extraction process, which on a volume basis are the most important products obtained from soybeans, have the de-fatted flakes as an intermediary product that requires further treatment.

1.2.1. Common Processing Methods

The various soybean products are obtained through the separation or extraction of the different components of the soybean. After being cleaned and dehulled, one of three processes is used to separate the soybean oil from the protein meal (this is also called "crushing" or "oil mill" operations). These processes are:

Solvent extraction: This process, which is the one used most commonly around the world, uses hexane to leach or wash (extract) the oil from flaked oilseeds. This method reduces the level of oil in the extracted flakes to one percent or less.

Continuous pressing: This process is performed at elevated temperatures, using a screw press to express the oil from ground and properly conditioned soybeans. The pressed cake is reduced to between 4 percent and 6 percent oil content by this method.

Hydraulic or batch pressing: This is an intermittent pressing operation carried out at elevated temperatures in a mechanical or hydraulic press after the soybeans have been rolled into flakes and properly conditioned by heat treatment. It is the oldest known method of processing oilseeds.

Solvent extraction is the most efficient and widely used process at present where non-polar solvents (commonly hexane and hexane isomers) are used to extract the oil. In the case of solvent extraction, the flakes are de-solventized. All flakes are toasted in order to eliminate the heat-labile anti-nutritional factors. Sometimes the hulls obtained in the preparatory steps are added back to the toasted flakes. This is done in variable degrees resulting in soybean meals with variable levels of fiber and crude protein. When no hulls are added, the higher protein meals are obtained. These are the meals used predominantly in poultry diets and aquaculture diets.

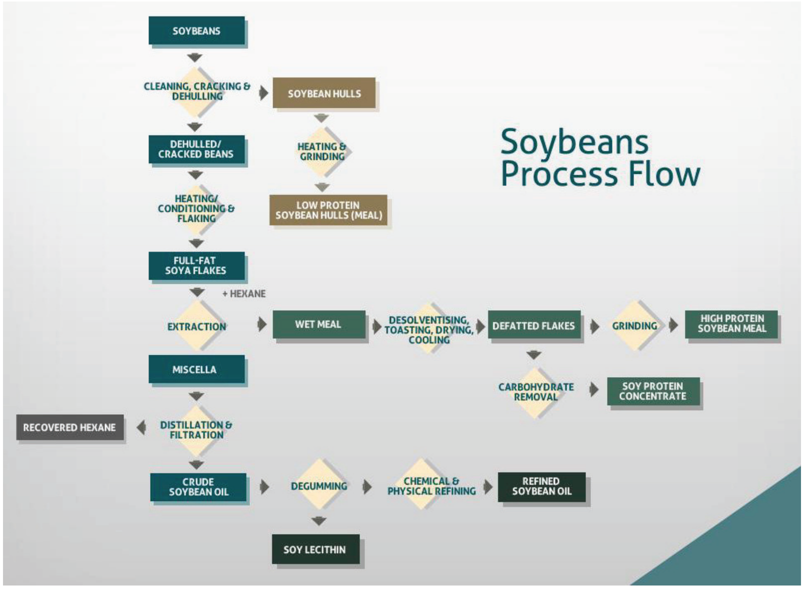


Figure 6. A schematic of the steps in soybean processing
Source: Wilmar International, Singapore

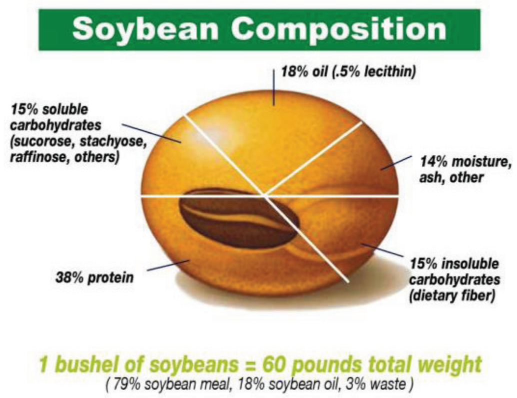


Figure 7. Typical composition of soybeans and conversions that can be used when estimating yields on meal and oil. *Source:* WISHH

Flash de-solvenization or heat vacuum drying of the de-fatted flakes produces the white flakes that are higher in protein quality (solubility) and do not have the undesirable darker color. Through a series of different extraction and precipitation processes, soy protein isolates (SPI) or soy protein concentrates (SPC) are produced. Whereas SPI production is fairly standardized, different methods of extraction are used to obtain the SPC resulting in slightly different compositional characteristics. SPC can be further elaborated (grinding, texturizing, separation on the basis of molecular weight) to obtain a large array of products used in human nutrition. SPI and SPC are used in animal nutrition but are limited to specialty diets due to their relatively high cost. New research initiatives are testing some of the SPC's in the high-protein aquaculture diets used on young fish and carnivorous fish species.

1.2.2 Definition and Application of Soybeans and Soy Products.

The number of soy products currently being used in the feed industry is large, and recent years have seen a dramatic expansion of specialty products based on soybeans. These evolved, value-added products may differ significantly among producers, with each producer applying proprietary knowledge and specialized treatments. Typically, value–added products must be evaluated on the basis of the entity that produces them taking into account the guarantees provided by the manufacturer or distributor. Consistent analysis of these producer-specific products allows classification and the building of an accurate database along with improved confidence about the product. This increased level of knowledge will allow an analysis schedule of decreased intensity and drive increased inclusion rates in diets.

Commodities as well as the value-added products can be classified in a specific class or group of products for which a sufficiently specific description can be developed. For an efficient and correct use - as well as a meaningful interpretation of analytical results - a precise and generally agreed upon definition of the product is needed. Trading, purchasing, formulation, and the entire operation of feed manufacturing depend on the precise referencing of a raw material and the consistent use of the correct name and description. Also the quality control mechanisms that have been introduced in the feed industry require a precise description and classification for all ingredients.

Although many databases and ingredient tables have their own classification system, the most widely recognized system is probably the IFN system (International Feed Name and number), according to Kearl *et al*., (1980) prepared for INFIC, (International Network of feed Information Centers). In this system, ingredients have been divided into eight fairly arbitrary feed classes on the basis of their composition and use (NRC, 1982). The system is widely used in the UK, the US and in Canadian feed composition tables but less so in other countries.

In the IFN system, ingredients are assigned a six digit code with the first digits denoting the International Feed Class Number. With the exception of soybean hay, soybean hulls (class), lecithin, soybean mill run and soybean mill feed (class 4), soy products listed in table 1 fall in the class of protein supplements (5) defined as products that contain more than 20 % crude protein on a dry matter basis. The five digits following the class number is the link between the INF and chemical and biological data in the USA databank (NRC, 1982). The number appears generally on official US ingredient specifications and, although the system may not be used by all feed producers or manufacturers, it provides an easy and systematic reference for quality systems and formulation purposes.

A brief and general description is available for many soy products. This description has the advantage of providing information that is not generally captured in compositional tables. It also provides for a general appreciation of the origin and quality and thus the potential applications or uses in a feed. Although these definitions might differ slightly between different sources, they are in general sufficiently similar to use them interchangeably. The Association of American Feed Control Officials (AAFCO) publishes at regular intervals reference specifications for soybean products (AAFCO, 2006). The definitions listed in table 1 have been used.

Table1. Description and Classification of Soybean Products most often used in animal feeds, in alphabetical order. Adapted from AAFCO Official Publication 2006 and Canadian Food Inspection Agency, 2003. The products listed in bold print are used in aquaculture feeds.

Full Fat Soybeans, Extruded (AAFCO: Ground Extruded Whole Soybeans) is the meal product resulting from extrusion by friction heat and/or steam of whole soybeans without removing any of the component parts. It must be sold according to its crude protein, fat and fiber content. IFN 5-14-005.

1. **Full Fat Soybeans, Raw (AAFCO:** Ground Soybeans) are obtained by grinding whole soybeans without cooking or removing any of the oil. IFN 5-04 -596.
2. **Full Fat Soybeans, Roasted (AAFCO:** Heat Processed Soybeans) is the product resulting from heating whole soybeans without removing any of the component parts. It may be ground, pelleted, flaked or powdered. It must be sold according to its crude protein content. Maybe required to be labeled with guarantees for maximum crude fat, maximum crude fiber and maximum moisture (CFIA 2003). IFN 5-04-597.

3. **Kibbled Soybean Meal** is the product obtained by cooking ground solvent extracted soybean meal, under pressure and extruding from an expeller or other mechanical pressure device. It must be designated and sold according to its protein content and shall contain no more than 7% crude fiber. IFN 5-09-343.
4. **Soy Flour** is the finely powdered material resulting from the screened and graded product after removal of most of the oil from selected sound cleaned and dehulled soybeans by a mechanical or solvent extraction process. It must contain no more than 4.0% crude fibers. Some organizations also require labeling guarantees for minimum crude protein and maximum crude fat and moisture. IFN 5-12-177.
5. **Soy Grits** is the granular material resulting from the screened and graded product after removal of most of the oil from selected, sound, clean and dehulled soybeans by a mechanical or solvent extraction process. It must contain no more than 4% crude fiber. Soybean grits mechanical extracted: IFN 5-12-176. Soybean grits solvent extracted: IFN 5-04-592.
6. **Soy Lecithin** or Soy Phosphate is the mixed phosphatide product obtained from soybean oil by a degumming process. It contains lecithin, cephalin and inositol phosphatides, together with glycerides of soybean oil and traces of tocopherols, glucosides and pigments. It must be designated and sold according to conventional descriptive grades with respect to consistence and bleaching. IFN 4-04-562.
7. **Soy Protein Concentrate** is prepared from high quality, sound, dehulled soybean seeds by removing most of the oil and water soluble non-protein constituents from selected, sound, cleaned, dehulled soybeans (CFIA 2003) and must contain not less than 65% protein on a moisture-free basis. It shall be labeled with guarantees for minimum crude

protein, maximum crude fat, maximum crude fiber, maximum ash and maximum moisture. IFN 5-32-183.

8. **Soy Protein Isolate** is the major proteinaceous fraction of soybeans prepared from dehulled soybeans by removing the majority of non-protein components, and contains not less than 90% protein on a moisture-free basis. The CFIA (2003) adds that the original material must consist of selected, sound, cleaned, dehulled soybeans and that it shall be labeled with guarantees for minimum crude protein (90%), maximum ash and maximum moisture. IFN Number 5-08-038 (CFIA lists this product with the IFN Number 5-24-811.

9. **Soybean Flour Solvent Extracted (or Soy flour)** is the finely powdered material resulting from the screened and graded product after removal of most of the oil from dehulled soybeans by a solvent extraction process. It shall contain less than 4 percent crude fiber. It shall be labeled with guarantees for minimum crude protein, maximum crude fat, maximum crude fiber and maximum moisture. IFN 5-04-593.

10. **Soybean Hulls** consist primarily of the outer covering of the soybean. IFN-1-04-560.

11. **Soybean Meal, Mechanical Extracted** is the product obtained by grinding the cake or chips which remain after removal of most of the oil from soybeans by a mechanical extraction process. It must contain no more than 7% crude fibers. It may contain an inert, non-toxic conditioning agent either nutritive or non-nutritive or any combination thereof, to reduce caking and improve flowability in an amount not to exceed that necessary to accomplish its intended effect and in no-case exceed 0.5%. The name of the conditioning agent must be shown as an added ingredient. IFN 5-04-600.

12. **Soybean Meal, Dehulled, Solvent-Extracted** is obtained by grinding the flakes remaining after removal of most of the oil

from dehulled soybeans by a solvent extraction process. It must contain no more than 3.3% crude fibers. It may contain an inert non-toxic conditioning agent either nutritive or non-nutritive or any combination thereof, to reduce caking and improve flowability in an amount not to exceed that necessary to accomplish its intended effect and in no-case to exceed 0.5%. The name of the conditioning agent must be shown as an added ingredient. IFN 5-04-612. It may also be required to be labeled with guarantees for minimum crude protein, maximum crude fat and maximum moisture (CFIA 2003).

13. **Soybean Meal, Solvent-Extracted,** is the product obtained by grinding the flakes which remain after removal of most of the oil from soybeans by a solvent extraction process. It must contain no more than 7% crude fibers. It may contain an inert, non-toxic conditioning agent either nutritive or non-nutritive and any combination thereof, to reduce caking and improve flowability in an amount not to exceed that necessary to accomplish its intended effect and in no-case exceed 0.5%. It shall contain less than 7 percent crude fiber. The CFIA (2003) specifies that it shall be labeled with guarantees for minimum crude protein, maximum crude fat and maximum moisture. IFN 5-04-604.

14. **Soybean Oil consists** of the oil from soybean seeds that are commonly processed for edible purposes. It consists predominantly of glyceride esters of fatty acids. If an antioxidant(s) is used, the common name or names shall be indicated on the label. It shall be labeled with guarantees for maximum moisture, maximum insoluble matter, maximum un- saponifiable matter and maximum free fatty acids. IFN 4-07-983.

The list in Table 1 demonstrates the large diversity of soy products and different methods of producing them. Although it only represents the major soy products produced, it provides a brief description of how the product is obtained and for some products a compositional reference point. The common name and IFN is provided which allows for a consistent and non-equivocal use of ingredients, important in quality systems. The description gives an adequate back ground of the products for trading and classification purposes, references in quality systems and production purposes. It is sufficiently precise to provide clear reference points for product definition and contract agreements but general enough to cover a substantial variation in composition and production processes. For proper use of an ingredient, additional analytical data should complement the information provided in the description. However, for analytical purposes the descriptions provide general back ground information as to what can be expected and how analysis should be carried out or what results may be expected. When considering formulation objectives, the description only serves as a classification aide and more precise compositional data will be necessary.

As previously mentioned, the products listed in Table 1 only represent the major soy products produced. At present, a large number of additional specialty products are marketed and the list does not adequately reflect the acceleration seen in the development of new soy products mostly branded products.

The most important products in terms of volume of use are soybean meals (SBM), solvent extracted, or dehulled (items 13 and 14, Table 1) resulting from the original use of soybeans i.e. the removal of oil. This is also the case for the mechanical extracted SBM (item 12) although this type of SBM is much less common. Full fat soybeans (FFSB; items 1,2,3) in raw, extruded or roasted form are defined and their use (particularly for the extruded and roasted forms) is increasing due to their high energy content, especially in formulations where animal products (meat and bone meals and fats) were previously of interest. One fiber-rich product is listed as soybean hulls (item 11). The interest in soybean hulls is important and an increasing application in the compound feed industry, especially for ruminants. It is rarely used in aquaculture diets, except for feeds formulated for the herbivorous grass carp (*Ctenopharyngodon idella*), popular in China.

Soy flour and soy grits are primarily products destined for human consumption although minor amounts may find an application in specialty animal diets. Technological modifications of these products have produced different types of flour and grits. They are further classified and commercialized according to their application objectives with the main differences being the level of fat content or heat treatment.

Soy lecithin is produced when the oil fraction is "de-gummed" and it has several human and animal feed uses. The three main phosphatides in commercial soy lecithin are phosphatidyl choline (also called "pure" or "chemical" lecithin to distinguish it from the natural mixture), phosphatidyl ethanolamine (popularly called "cephalin"), and phosphatidyl inositol (also called inositol phosphatides). Commercial soy lecithin also typically contains roughly 30%-35% unrefined soy oil. Because it's readily available from plentiful soybean crops all over the world, it's the cheapest and easiest type of lecithin to mass manufacture. Lecithin is used as an emulsifier in many types of human food products and animal feeds and is often added to fish feed formulations in small doses.

With the increased complexity of production processes aimed at removing ANF and improving protein digestibility, a clear understanding of the products and the production process becomes more important. Quality difference between producer/suppliers for these products can be substantial, especially for the more evolved products. These differences need to be verified and understood at the feed manufacturer's level. It remains the responsibility of the user to carry out the needed quality analysis and classify suppliers and products accordingly. Reliable manufacturer's information is, of course, important but verification remains the basis of this tool and of the overall quality assurance program. The quality of the information provided by the manufacturer must be an integral part of the "supplier classification process".

The quality of ingredients plays a determining role in the level at which these ingredients are used in animal diets. Quality criteria used to determine the inclusion level for an ingredient go beyond the standard nutrient levels, and have often more to do with residual ANF, storage and contamination and the physiological characteristics of the animal. The inherent variation in quality and chemical characteristics associated with these ingredients make repeated quality analyses necessary which in turn will determine more precisely the inclusion levels employed. The nutritionist's experience and interpretation of the quality analyses play a major role in defining the final inclusion level used in particular diets. Table 2 gives estimates of maximum inclusion levels of each product under practical conditions of diet formulation. The inclusion levels suggested are for inclusion in complete diets and are thus necessarily general. The levels need to be further defined for each feed manufacturer, the manufacturing process and the feed being formulated. Some of the maxima suggested are not defined by any inability of the animal to use the nutrients in a given product, but rather by the effects of specific nutrients on carcass or product quality. Such is the case for extruded or roasted FFSB or soybean oil. Other maxima are controlled by economic considerations. While higher inclusions in diets may be possible, the added cost of increasing those levels needs to be evaluated.

Table 2. Application of soybean products as presented in the 2nd Edition of Manual of soy quality analyses by U.S. Soybean Export Council (ASA-IM)

PRODUCT	SPECIES					LEVEL %
	Poultry	Swine	Ruminant	Aqua	Pet	
Condensed Soybean			X			10
Dried Soybean			X			15
Ground Extr. Whole	X	X	X	X	X	3[5]
Ground Soybean Hay		X	X			20
Ground Soybeans		X	X			15
Heat Processed	X	X	X			15
Kibbled Soybean	X	X	X	X	X	10 (Y)
Soy Lecithin Or Soy	X	X	X	X	X	3
Soy Protein	X	X	X	X	X	7 (Y)
Soy Protein Isolate	X	X	X	X	X	10 (Y)
Soybean Feed, Solv.	X	X	X	X	X	5 (Y)
Soybean Flour	X	X	X			40
Soybean Hulls	X	X	X			25
SBM Mech Extract	X	X	X			30
SBM Dehulled Solv.	X	X	X	X	X	35
SBM Solv. Extract	X	X	X	X		35
Soybean Mill Feed	X	X	X	X		10
Soybean Mill Run	X	X	X	X		10
Soybean Oil	X	X	X	X	X	8

[1]Suggested upper-use levels in diets of different domestic species; this will vary with age of animal, quality, composition and analysis of product; does not include young animal diets unless specifically indicated. Detailed and extensive analyses will allow discretionary changes in usage level.

[2]Species: Production diets (growing/finishing) for Poultry, Swine, Ruminants, Aqua (salmonids); Pets (dogs).

[3]On a diet dry matter basis. “Y” indicates primarily in young animal diets.

[4]Higher levels may be used in salmon and trout grower, finisher diets.

[5]Maximum inclusion of oil in Ruminant diets should not exceed 2%.

1.3. Chemical and Nutritional Composition of Soybean Products.

The compositional data provided in the Tables 3 and 4 are better descriptors of the nutritional characteristics of soybean products.

The table values provide means based on a large number of samples covering many years and a wide range in origin. They cannot be used as standard values but only as reference points around which analysis of individual samples should be situated if they are to be identified by the specific ingredient name.

For most users of soy products the detailed nutrient concentrations serve as a basis to formulate diets and to calculate total nutrient supply to animals. However, for precise formulations the analytical data on the ingredient in the plant should be used. The use of the table values, especially because of the large contribution that soy products make to the protein and amino acid supply, may lead to significant variations in nutrients between the formulated value and the real diets.

The compositional data in Table 4 includes nutrients and ANF that can be directly analyzed in a large and well equipped laboratory. Routine analyses, as carried out in standard quality control procedures or smaller laboratories, mainly concern the proximate analysis, the Van Soest fiber components (with the exception of lignin) and the minerals calcium and phosphorus. These analyses (especially the proximate) are most often used to derive other nutrient values such as amino acids or energy. In advanced formulation systems they are generally combined with estimates of digestibility for each individual nutrient. No digestibility data are included here as this information is not necessarily the result of direct observations but rather of literature compilations and research conducted by feed compounders. Thus digestibility data used in formulation systems can differ considerably among users and are generally considered proprietary information.

Table 3. Composition of major soy protein ingredients used in animal feeds.[1,2,3]

	Unit	Full fat soybeans, roasted	SBM mechanical extracted	SBM solvent extracted 44	SBM solvent Extracted 48	SBM solvent extracted 50	Soybean hulls	Soy protein concentrate	Soy protein isolate
International feed number		5-04-597	5-04-600	5-04-600	5-04-604	5-04-604	1-04-560	5-32-183	5-24-811
Dry matter	%	89.44	89.80	88.08	87.58	88.20	89.76	91.83	93.38
Crude protein	%	37.08	43.92	44.02	46.45	48.79	12.04	68.60	85.88
Crude fiber	%	5.12	5.50	6.26	5.40	3.42	34.15	1.65	1.32
Ether extracts	%	18.38	5.74	1.79	2.13	1.30	2.16	2.00	0.62
Ash	%	4.86	5.74	6.34	6.02	5.78	4.53	5.15	3.41
NDF[4]	%	12.98	21.35	13.05	11.79	9.95	56.91	13.50	-
ADF[4]	%	7.22	10.20	8.76	7.05	5.00	42.05	5.38	-
ADL[4]	%	4.30	1.17	0.75	0.90	0.40	2.05	0.40	-
Starch	%	4.66	7.00	5.51	5.46	3.28	5.95	-	-
Total sugars	%	6.70	9.76	9.06	9.17	9.29	1.40	-	-
Gross energy	kcal/kg	5013	-	4165	4130	4120	3890	4280	5370
Lysine	%	2.34	3.50	2.85	2.89	3.00	0.73	4.59	5.26
Threonine	%	1.53	2.21	1.80	1.84	1.90	0.73	2.82	3.17
Methionine	%	0.52	0.80	0.62	0.63	0.67	0.14	0.87	1.01
Cystine	%	0.55	0.77	0.68	0.73	0.73	0.16	0.89	1.19
Tryptophane	%	0.49	0.74	0.56	0.63	0.65	0.12	0.81	1.08
Calcium	g/kg	2.62	2.96	3.12	3.07	2.68	4.96	2.37	1.50
Phosphorus	g/kg	5.70	6.64	6.37	6.37	6.36	1.59	7.63	6.50
Magnesium	g/kg	2.80	2.84	2.72	3.03	2.88	2.23	1.85	0.80
Potassium	g/kg	15.93	20.28	19.85	22.00	20.84	12.15	12.35	2.75
Sodium	g/kg	0.29	0.33	0.18	0.18	0.88	0.10	0.55	2.85
Linoleic acid – C18:2	%	9.70	2.87	0.64	0.80	0.56	1.21	-	-

[1]All values as fed basis

[2]SBM=soybean meal.

[3]Source: compilation of NRC, INRA-AFZ, CVB, FEDNA and selected supplier

[4]NDF = neutral detergent fiber; ADF = acid detergent fiber; ADL = acid detergent lignin (klason lignin)

Source: Van Eys, Manual of quality analyses for soybean products in the feed industry, 2nd ed

Table 4. Analytical characteristics of common types of soy protein products. [1,2]

Product type	Unit	Soybean seeds, raw	SBM	Enzyme treated SPC	Alcohol extracted SPC	SPI
Moisture	%	10 – 12	10 – 12	6 – 7	6 – 7	6 – 7
Crude protein	%	33 – 37	42 – 50	55 – 60	67 – 70	>85
Fat	%	17 - 20	0.9 – 3.5	2.5	0.5 – 3.0	0.1 – 1.5
Ash	%	4.5 – 5.5	4.5 – 6.5	6.2 – 6.8	4.8 – 6.0	2.0 – 3.5
Oligosacharides	%	14	15	<1.0	<3.5	<0.4
Stachyose	%	4 – 4.5	4.5 – 5	<0.3	1 - 3	<0.2
Raffinose	%	0.8 – 1	1 – 1.5	<0.2	<0.2	<0.1
Verbascose	%	-	0.3 – 0.4	-	-	-
Trypsin inhibitors	mg/g	25 – 50	1.6 – 5.0	1 – 2	2 – 3	<1
Glysinin	mg/g	150 – 200	20 – 70	<0.01	<0.1	<0.01
B-conglycinin	mg/g	50 – 100	3 – 40	<0.01	<0.01	<0.005
Lectins	ppm	2100 – 3500	20 – 600	<1.0	<1.0	<1.0
Saponins	%	0.5	0.6	0	0	0
Phytic acid bound	%	0.38	0.42 – 0.49	0.6	0.6	-

Note. SBM = defatted soybean meal; SPC = soy protein concentrate; SPI = soy protein isolate

[1]Adapted from: Hansen (2003), Peisker (2001), Fasina et al (2004), Maenz et al (1999), CVB (1999), Ruiz (2011).

[2]All values as fed basis

Source: Compilation by Van Eys, Manual of quality analyses for soybean products in the feed industry, 2nd ed.

While a large number of compositional tables and publications for soybean products exist, those data cannot be considered as standard values, especially not for trading purposes. For trading and contractual purposes they are too detailed and thus unpractical. Furthermore, they do not provide the required borderline minimum or maximum values for limited number readily identifiable parameters.

A limited number of official standards have been published to start with the basic material: whole, untreated soybeans or seeds (IFN 5-04-610). As is the case for all other grains and seeds the USDA publishes official standards for soybean grains as defined under the United States Grain Standards Act. These standards do not generally change much over time and under the act soybeans are defined as grains that consists of 50 percent or more of whole or broken soybeans (Glycine max (L) Merr.) that will not pass through an 8/64''round hole sieve (3183 microns) and does not contain more than 10.0 percent of other grains for which standards have been established under the United States Grain Standards Act (USDA, 2007).

For trading purposes – especially in view of specific applications and export requirements – additional specifications are provided by dividing soybeans into classes and grades. Only two classes of soybeans have been defined (yellow soybeans and mixed soybeans) but 5 grades are specified. The grades and grade requirements for the major export countries (USA, Brazil and Argentina) are similar. However, while Brazil and Argentina have a special export grade, the United States does not define a specific export grade as soybeans are exported from the US at any pre-defined specification or grade. The USDA (2007) description of grades is provided in Table 5.

Next to whole soybeans, only three soybean products (two soybean meals and soybean oil) have standard values. Used as official reference standards, they have been developed by the National Oil Processors Association (NOPA, 1999, 2011) and are also published by the American Soybean Association (ASA, 2005) in the Soy Importers Guide. These standards are now widely accepted and provide minimums or maximums on only a few, easily identifiable, key parameters. In the case of soybean meals, their main purpose is the classification of the soybean meal products into two main categories: solvent extracted SBM and de-hulled, hi-pro, SBM.

For soybean oil, the NOPA standards refer to crude de-gummed soy bean oil mainly with food application purposes in mind. These standards serve as a general guide for transactions, thus assuring a minimal degree of quality and consistency in at least the three main types of soy products being traded. However, the standards and trading guidelines proposed by NOPA are not binding.

Table 5. U.S. grades and grade requirements for soybeans.

Grading factors	**Grades U.S. Nos**			
	1	2	3	4
Damaged kernels: - Heat (part of total) - Total	 0.2 2.0	 0.5 3.0	 1.0 5.0	 3.0 8.0
Foreign material	1.0	2.0	3.0	5.0
Splits	10.0	20.0	30.0	40.0
Soybeans of other colour[1]	1.0	2.0	5.0	10.0

	Maximum count limits of			
Other materials: - Animal filth	9	9	9	9
Castor beans	1	1	1	1
Crotalaria seeds	2	2	2	2
Glass	0	0	0	0
Stones[2]	3	3	3	3
Unknown foreign substance	3	3	3	3
Total[3]	10	10	10	10

U.S. sample grades are soybean that:

a) Do not meet the requirements for U.S. Nos 1,2,3, or 4; or
b) Have a musty, sour, or commercially objectionable odor (except garlic odor); or
c) Are heating or otherwise of distinct lower quality.

[1]Disregard for mixed soybeans

[2]In addition to the maximum count limit, stones must exceed 0.1 percent of the sample weight

[3]Includes any combination of animal filth, castor beans, crotalaria seeds, glass, stones, and unknown foreign substances. The weight of stones is not applicable for total other material. (USDA, 2007).

These standards principally serve the trading and marketing of US soybean products within the USA but due to their wide acceptance, their impact goes well beyond US meals (and oils) as they are generally applied to compare and benchmark soybean products from other origins.

Solvent extracted soybean meal can be the result of blending back soybean hulls in the dehulled meal. The blending of different types of soybean meals or soybean components at the point of shipping is allowed under NOPA regulations and standards for minimum blending procedures are provided. As a matter of fact, this can be the source of a significant variation in quality and chemical composition. However, blending of soybeans is not permitted. For soybean meals, only soy hulls, soybean mill run and soybean mill feed are permitted to be blended with soybean meals before the point of sampling. The blending must lead to a meal of uniform quality representative of the contract terms.

Dehulled soybean meal is of particular interest to fish feed producers because most fish feed formulators seek to keep fiber as low as possible. One of the challenges with using plant protein is the typically high fiber associated with these ingredients.

Table 6. Specifications for solvent extracted and dehulled soybean meal (%).

	Min/Max	Solvent extracted SBM	Dehulled SBM
Moisture	Max	12	12
Protein	Min	44	47.5 – 49.0
Fat	Min	0.5	0.5
Crude fiber	Max	7	3.3 – 3.5
Anti-caking agent	Max	0.5	0.5

Source : (National Oilseed Processor Association (NOPA), 2011)

The National Oil Processor Association (NOPA) in the U.S. sets quality standards and describes the various processed products derived from soy. The NOPA quality standards applied to domestic shipments are also followed for international shipments although contracts may be patterned after North American Export Grain Association (NAEGA) or the Grain and Feed Trade Association (GAFTA). This ensures quality and customer satisfaction.

For SBM, the NOPA standards clearly aim at providing a minimum number of primary quality characteristics and as such are only a basis for contract specifications (Table 6). Meals purchased under NOPA contract specifications will therefore still need additional analysis. In order to provide greater quality assurances and meet the nutritional requirements of the feed compounder or nutritionist, additional recommendations have been added by NOPA (Table 7).

Table 7. Recommended additional specifications for soybean meal.

Ash	<7.5%
Acid insoluble ash (silica)	<1%
Total lysine	>2.85% (basis 88% dry matter)
Digestible lysine	Equal or >88% of total lysine
Protein solubility in 0.2% KOH	78-85% (or more if urease is within specifications
Protein dispersibility index	15 – 40%
Urease activity	0.02 – 0.30 pH unit rise
Trypsin inhibitors	< 4 mg/g of meal
Bulk density	57 – 64 g / 100 cc
Screen analysis (mesh)	95% thru #10 mesh, 45% thru #20 mesh, 6% thru #80 mesh
Texture	Uniform, free flowing, no lumps, cakes, dust
Color	Uniform particle colors of light tan to light brown
Odor	Fresh, not musty, not sour, not like ammonia, not burned
Contaminants	Free of urea, free of ammonia, free of Mycotoxins and molds, free of pesticides, grains, and seeds

These are only recommendations that apply in a non-binding manner to all soybean meals. Rather than guidelines, they should be regarded as further suggestions to both producers of soybean meal and buyers, provided in an effort to improve the quality of US soybean meals. Under practical conditions there remains a large variation around these recommendations and from a feed compounder's point of view, information on quality requirements for SBM needs to be still more detailed. Also, new parameters have been added and more recently evaluations have changed slightly. For instance there is a definite tendency for KOH values to shift to the high end of the established range (close to the 85 % value).

Soybean meal has one of the best amino acid profiles relative to other vegetable –protein meals. Relative to fishmeal, soybean meal is rich in lysine, but deficient in methionine and cysteine. Utilization of soybean meal by fish is variable, but with adequate oil, mineral, and possible amino acid supplementation, properly toasted soybean meal is capable of replacing much of, if not all, the fish meal in diets for many aquaculture species. Soybean meal is readily available in most world markets at 25 to 50% of the cost of fish meal, but it contains 66 to 75% of the crude protein found in fishmeal. In general, soybean meal is much more uniform and standard, compared to fishmeal. Soybean meal is traded on the basis of weight, moisture, protein, fat and urease level.

References

Please find all references from Chapter VI A at the end of Chapter VI B.

CHAPTER – VI B

SOYBEAN PRODUCTS IN FISH DIETS

Author
Karen L. Veverica
Director, E.W. Shell Fisheries Center
School of Fisheries, Aquaculture and Aquatic Sciences
Auburn University, AL USA

2. SOY IN AQUACULTURE DIETS

Aquaculture diets in the early 1900's up until about the 1960's relied on fish meal and other animal protein sources. Many fish farmers used fish meal or "trash fish" in their first prepared diets and some still do. The fish sources have often been species that were considered unfit for human consumption such as anchoveta and menhaden. The poultry industry also depended on inexpensive fish meal as a good source of protein and energy. However, as fish meal and fish oil use increased for both human and animal consumption, and catches remained stagnant, the price of fish meal and oil began to climb. Furthermore, the act of feeding fish to fish seemed environmentally and ecologically unsustainable and unwise. But most importantly, given the current state of increase in aquaculture production and the present levels of fishmeal and fish oil used in aquaculture diets, it is estimated that the needs for these products in aquaculture diets will surpass the total availability. Therefore, reducing fish product levels in aquaculture diets is the only way that production of fish feeds can increase to serve the needs of aquaculture production. (NRC 2011).

Choices for replacing fish products are: use of animal processing byproducts, use of plant protein and oil sources and use of single-cell proteins and oils. Therefore, the study of plant protein and oil sources remains one of the most active areas of aquaculture research. Plant protein products now commonly used to replace fish meal in fish feeds are full fat roasted soy, solvent extracted soybean meal, cottonseed meal, rapeseed (canola) meal, and corn and wheat gluten meals and brewers' yeast. Soybean protein products are the most suited and commonly used alternative plant protein sources in aquaculture diets due to their amino acid profile and relatively high coefficient of digestibility for most species. However, many fish diets use a combination of two or more plant protein sources. Progress in the understanding of fish nutritional requirements, especially in terms of essential amino acids, and in the processing of soy to isolate the protein and reduce anti-nutritional factors (ANF's) has contributed to the steady rise in use of soy as a component of fish feed.

For the purpose of feed formulations, fish can be divided into 4 main nutritional groups: omnivorous freshwater fish (these are mostly warmwater species); carnivorous non-salmonids, salmonids, and marine fish. Delbert Gatlin III (2002) has reviewed the use of soy products and potential in the diets for key omnivorous species of freshwater fish for the United Soybean Board (USB) and the American Soybean Association (ASA). This and other papers that cover marine fish, salmonids and marine shrimp can be viewed at www.soymeal.org. According to Gatlin, the use of soybean meal-based diets for freshwater omnivorous fish is well underway and its expansion will help increase production in high growth areas such as Southeast Asia and the Indian subcontinent.

2.1. Replacing fish meal

Fishmeal can be replaced by proteins of plant origin or with land-based animal products. Use of animal products in fish feeds was banned by the European union following an outbreak of bovine spongiform encephalopathy in the UK in the late 1980's but the ban has been relaxed. Some of the blood meals are now allowed. But there is still some consumer resistance to the use of animal byproducts in animal feeds, including fish feed. Use of plant proteins are much more acceptable to consumers but even these encounter resistance due to perceived risks of genetically modified plants, presence of allergens and increased rainforest removal to provide additional farming land.

Digestibility coefficients of soybean meal range in the 90% and above for the freshwater omnivorous species, thus making soybean products in the range of fish meal. Summaries of research to replace fish meal with soy and other vegetable protein sources have been published by Lim and Domini (1989) Gatlin (2002), Nguyen (2008), and Lim et al. (2008).

Freshwater omnivorous fish have been the easiest group for which to formulate feed using high percentages of plant protein. Early research into maximum recommended levels of soybean began in the 1970's with research on carp, (Viola, 1975), catfish (Cruz, 1975; Saad, 1979) and tilapia (Davis and Stickney, 1978). Brown and Smith (2002) have summarized the findings with respect to soy and produced a Fact Sheet printed by the Soybean meal Information Center and in the soybean meal newsletter. In it they list the current 54 aquaculture species that have been fed soybeans. Although the freshwater omnivores are the easiest to feed with high levels of soybean meal in the diet, it is clear that at least 10 to 15% soybean meal is tolerated by virtually all of the fish.

The variety of experimental designs has made it difficult to interpret many of the results of experiments conducted on inclusion of soybean and soybean meal in fish feeds. Some early research merely replaced fish meal with soybean meal and tried to maintain uniform protein levels with no regard to amino acid requirements.

Reasons for poor performance were often merely speculated, without further research to validate the reason. Data on digestibility of soybean meal are obtainable for several species but there is very little data on the availability of the individual amino acids so further studies are needed in order to further refine the diets using soy and other plant protein sources for other species. Gatlin (2002) could find only three aquatic species for which the amino acids availability of soy products were tested: tilapia, channel catfish and silver perch.

Total replacement of fishmeal is possible even in high protein diets using dehulled solvent-extracted soybean meal coupled with soy protein concentrate or other plant protein concentrates, and with crystalline amino acids but it may not make practical sense at this time given the price of such ingredients. As feed-grade protein concentrates become available, this may change.

Soybean products now typically make up 25 to 55% of the feed ingredients in diets for the freshwater omnivorous species of fish like tilapia and the various carp species. The salmonids and many marine species are more challenging when it comes to replacing fish meal with plant protein sources, but progress is being made.

2.2. Replacing fish oil

Diets have been made using plant proteins and the associated oils for warmwater, omnivorous fish without reducing growth. A diet using full-fat soy or pressed soy to satisfy the lipid needs that are typically 3 to 12% depending on fish size, with additional protein provided by solvent-extracted soybean meal has been demonstrated (Lim 1992). Fish oil is not required for nutrition in this group of fish species but it does increase the palatability of an all-plant protein feed.

However, marine fish and salmonids show a high degree of dependence on the n-3 long chain fatty acids and no suitable replacements have been found. Plants are notably deficient in omega 3 long chain fatty acids, which are found in marine fish, crustaceans and algae. These lipids have substantial health benefits to humans and are one of several reasons given by health professionals as to why people should consume more fish. Fish fed diets low in omega 3 lipids tend to also be low in omega 3 fatty acids and this is why the freshwater omnivores are said to be "less health alternatives" compared to the high-fat marine species.

Soy lecithin, removed during the degumming process of soybean oil is an excellent source of phospholipids, or phosphatides, for aquafeeds. Phospholipids are the molecules that make up cell membranes. It is used to improve protein and fat digestibility and aids in the pelletizing process and is generally included at 1 to 2% of the diet.

2.3. The soy in aquaculture program

For more than two decades, the American Soybean Association International Marketing program has sought to increase awareness of soy-based aquaculture diets by conducting more than 175 demonstrations of soy-based feeds. First efforts began in China in 1992 and have resulted in dozens of reports on new methods of fish production as well as new feed formulations and feeding methods. The high intensity production methods such as low volume-high density cages, and more recently, in-pond raceways require complete and well-formulated feeds, and the soy-based feeds have performed well. Improved less-intensive methods of pond aquaculture known as the 80:20 method using high quality feed have proven to be more profitable than using the low quality inputs previously used. As a result, China began using soybean meal and other soy products in fish feeds as well as in other animal feeds. Now, China consumes more than 1,400 metric tons of soy just for aquaculture (USSEC 2013). Efforts expanded to SE Asia, the Indian subcontinent the Middle East and Latin America.

Often, when a soy awareness program starts, the first feeds are made in the US and then shipped to the country if there is no feed mill yet capable of making the floating feeds. In countries where there are already fish feeds being manufactured, the mills are contracted to produce a soy-based feed to the project's specifications.

2.3.1. Results from Pakistan

Typically, first demonstrations of improved fish feeds seek to compare local practices with improvements that are already widely practiced elsewhere in the world. According to the brochures distributed by the FEEDing Pakistan project of ASA/WISHH, the advantages of using soy-based, extruded, floating fish feed (SBEFF) , compared to the traditional powdered diets and a similar mix that has been pelletized are:

- Fish grow bigger and faster with soy-based, extruded, floating feeds
- SBEFF improves feed conversion ratios (FCR)
- SBEFF reduces overall cost of production
- Utilization of SBEFF allows for shorter growing seasons
- Shorter growing seasons reduce direct labor costs
- Shorter growing seasons reduce costs of inputs
- Shorter growing seasons allow for multiple harvests per year

Farmers are encouraged to examine how an improved feed conversion justifies the higher price of the feed. Table 8 below was constructed using fish production data from trials on farms.

Table 8. Feed Cost Analysis for trials conducted in Pakistan

Feed Type	Cost / Ton	FCR Achieved	Feed Required (KG/MT)	Total Feed Cost to Rear 1000 KG
Mixed Ingredients	$400	4:1	4,000	$1,600
Mixed Ingredients-Pelleted	$500	3:1	3,000	$1,500
Milled Ingredients-Extruded	$800	1.7:1	1,700	$1,360
Milled Ingredients-Extruded w/US standard soybean	$840	1.6:1 1.4:1 1.2:1	1,600 1,400 1,200	$1,344 $1,176 $1,008
All figures in USD. Source: USDA GAIN Report Pakistan - Feb. 7, 2013				

The soy-based feed used in these trials contained about 50 percent soybean meal. Other results of soy-based extruded fish feeds manufactured in Pakistan are discussed in Chapter 12 on economics. The feed locally manufactured in Pakistan in 2013 used a 30% SBM in formulation with 5% fishmeal and 5% canola meal.

Picture: 2012 Feeding Trials, harvesting tilapia at Himalaya Fish Farms

Picture: 2012 Feeding Trial harvesting tilapia before farmers at Himalaya Fish Farms, Murdkee. Photo courtesy; R.S.N. Janjua, SoyPak

2.3.2. Results of trials and demonstrations conducted around the world

When shrimp farmers in Venezuela tested some soy-based feeds, (USSEC, 2011) they immediately saw the increased profitability and changed their shrimp feed formulations before the results were even published. They found that replacing 75% of the fishmeal in the diets with soybean meal reduced feed costs by $255 per metric ton and at no decrease in growth of the shrimp. In fact the shrimp fed the 75% fish meal replacement diet were slightly larger than those fed the commercial formulation and those fed the 50% fishmeal replacement formula.

Fishmeal tends to be used at very high rates in Africa, due to lack of fish feed formulation expertise at the small-scale, local level. Demonstrating successful production with soy-based feeds will assist beginning feed manufacturers and growers in selecting cost-effective feeds for tilapia and clarias production. It can also help feed producers examine other options because locally-sourced fish meal can be of poor quality and has been implicated in losses to tilapia farmers feeding diets made with contaminated fish meal or fish meal high in peroxide.

Table 9 below provides the formula that was used on a 32% protein demonstration feed produced in the US and shipped to Ghana. The

36% protein formulation for the fingerling stage contained 5% fish meal because this proved to be less expensive than using crystalline methionine to balance the amino acids.

Table 9. Formula used for demonstration floating fish feed in Ghana

LP code: GHANA32/06

Description	Lbs	%
Wheat-Ground	437.29	22.03
Wheat Midds 27-34% N	198.5	10
Rice Bran-Hifat	198.5	10
Soybean Meal 48%	941.62	47.44
Corn Gluten Meal, 60	99.25	5.0
Ethoxyquin Dry	0.4	0.02
Yelkinol Lecithin	19.85	1
Stay C 35%	1.49	0.07
Phosphate - Dicalcium	66.46	3.35
Fish Vitamin Premix	3.97	0.2
Fish Trace Mineral P	4.96	0.25
Fish Oil Downstream	41.57	2.09
Moisture Change	-28.86	-1.45
Total:	1985	100

Finished FEED 32/06

Nutrient	Units	Actual
[Volume]	%	100
Protein	%	32
Fat	%	6
Ash	%	8.12
Ndf	%	10.63
Moisture	%	10
Total Starch	%	15.98
Fiber	%	3.81
Calcium	%	1.04
Phosphorus	%	1.34
Available P	%	0.6
Methionine	%	0.51
Met + Cys	%	1.08

There are dozens of demonstrations and trials conducted in China, on a large variety of aquatic species. Red tilapia fed a soy-maximized all plant protein feed (Table 10) that was formulated to have 32% crude protein and 6% crude fat, with soybean as the main protein source, yielded an FCR for the red tilapia of 1.5. Average net return was $2,249/ha, giving a return on investment of 33%.

Filter-feeding silver carp was the secondary species, and combined yield averaged 8,048 kg/ha: 7,260 from the red tilapia and 788kg/ha from the silver carp (Cremer *et al*. 2004). This trial was run in Beijing, when water temperatures were 24-25C.

Other trials conducted in China used feeds prepared with high levels of soybean meal but small amounts of fish meal (typically 3 to 6% of diet) because this proved to be less expensive than using crystalline methionine to balance the amino acids. Trials conducted on grass carp, which is a fish that feeds on macrophytes and other plant matter used a soy-based feed that included soy hulls. Soy hulls are not typically used in aquaculture feeds, but they are a good source of digestible fiber and have been found to be a cost-effective fiber source for use in grass carp feeds. (Cremer et al. 2001)

Table 10. Formula for the ASA 32/6[1], soy-maximized feed used in the 2004 red tilapia demonstration feeding trial near Beijing, China. Fwusow/Xiamin feed mill in Fujian Province produced the feed in extruded, floating pellet form.

Ingredient	Percent of total
Soybean meal 47.5%	52.8
Wheat, SWW	23.2
Wheat middlings	10.0
Corn gluten meal 60%	6.0
Fish oil	3.5
Soy lecithin	1.00
Ca phosphate mono	2.70
Vit PMX F-2	0.50
Min PMX F-1	0.25
Stay C-35%	0.03
Ethoxyquin	0.02
Total	100.00

[1]The numerical component of the feed description refers to the percentage of protein and lipid, respectively, in the ration, i.e. 32/6 indicates 32% crude protein and 6% crude lipid.

After initial demonstrations showed how soybean meal could replace virtually all of the fish meal in diets for growing out juvenile to market size fish, trials began with the more specialty feeds for fry and fingerlings. These feeds typically have protein content above 40%, which is hard to make with even the Hi-Pro soybean meal. A low antigen animal feed grade SPC (65% protein) was used to replace all fish meal in diets for growing tilapia from 0.5 to 100 g in 67 days at water temperatures 30-32 C in Hainan, southern China (Enhua *et al*. 2010).

Cremer *et al.* (2008) reported on trials using soy protein concentrate (SPC) to replace fish meal portion of high soy diets that had been developed for the region for the fingerling production of common carp, grass carp, tilapia and channel catfish. There were no differences in fish growth, final harvest weight, FCR and survival. They concluded that SPC, supplemented with methionine can replace the fish meal and it can be economically viable to do so; if not now, then in the near future as fish meal availability may be limited. This will provide feed millers with more options for the high-protein fry and fingerling diets.

Non-salmonid marine finfish appear to tolerate higher level of dietary soybean protein products than salmonids, but lower than freshwater, warmwater omnivores such as channel catfish, tilapia, and carp. The ability of non-salmonid fish to utilize soybean protein products appears to vary among the types and quality of soy products, fish species, and fish size.

Solvent-extracted, dehulled, toasted SBM as an alternative protein source is one of the first fish meal replacements evaluated and remains a desirable ingredient for a variety of reasons. It has therefore become the dominant source of crude protein in what has become the channel catfish formulations, tilapia feed formulations, feeds for various types of carps and is present in almost all diets for omnivorous freshwater fish.

2.4. Outstanding Areas of Study

2.4.1. Overcoming the low methionine: soy-based fish feeds are typically limiting in methionine and sometimes lysine as well. For tilapia, it has been demonstrated that low-protein diets exhibit a slight deficiency but as the protein level increases (probably beyond the actual protein requirement), the deficiency is no longer exhibited. (Davis and Stickney, 1978; Enhua *et al*., 2010). However, for young fish, the protein requirement is higher, and once again, the feed formulator is faced with the choice of either adding supplemental crystalline amino acids to the various plant protein concentrates or using fish meal. Supplementation with crystalline amino acids has shown the limitations can be overcome but results are not as good as protein-bound amino acids. This is probably because the crystalline forms become available immediately, whereas the protein-bound forms are absorbed more slowly as the proteins are broken down.

2.4.2. Increased utilization/ digestibility to reduce environmental impacts (P digestibility; non-starch polysaccharides): the vegetable proteins tend to have their P bound up in phytate, which is relatively undigestible to fish, therefore, mineral sources of P are added. The mineral sources of P add some to the cost of the feed but the main problem is the P that is excreted can then contribute to excessive fertility in the surrounding waters. This is why several countries now have regulations on maximum P in effluents from aquaculture enterprises. Research to reduce the use of mineral P and increase the digestibility of phytate-P has centered around the use of phytase of fungal origin added to the feed. Phytase is heat labile, so top-coating has been recommended. Recent progress has been made on heat-resistant phytase but not all sources are the same. The phytase problem is not important in countries like Pakistan where tilapia are grown in ponds that are fertilized with diammonium phosphate. Other undigestible products in soy-based feeds are the non-starch polysaccharides. Sources of exogenous enzymes are being studied so as to further reduce pollutions from undigested feed and to increase the energy available to the fish.

2.4.3. Addressing ANFs and phytoestrogens: All plant substances have some type of anti-nutritional factor (NRC 2011). Hart and Brown (2005) present a short description of the outstanding areas of study for soybeans with respect to some of the concerns on anti-nutritional factors and phytoestrogen content of soybean. Because the omnivorous freshwater fish, like tilapia, were not as affected by the ANF's, rainbow trout and Atlantic salmon were used as test subjects by a group of universities collaborating on evaluating the ANF's.

Phytoestrogens were thought to either prevent successful spawning or possibly be carried over into the fish flesh and have some effect on human health. Neither of these could be demonstrated conclusively to be the case.

As for the anti-nutritional factors, these can be reduced by further concentrating the protein fraction of the soy by removing the soluble carbohydrates through extraction with aqueous alcohol to produce the soy protein concentrates. Some of the ANF's found in soybean meal that negatively affect fish species by causing gastrointestinal disturbances, intestinal damage and increased disease susceptibility are lectins, trypsin inhibitors, glycinin, B-conglycinin, saponins, and oligosaccharides. These are also documented to be greatly reduced in SPC, as compared to soybean meal. A further thermal processing produces what is called a low-antigen SPC that has been used in the high-protein shrimp and marine fish feeds as well as the fry and fingerling feeds for freshwater fish.

There is some suspicion that the non-starch polysaccharides are not only unavailable but are detrimental to the fish. Other research has indicated that some of the perceived ANF's actually improved the quality of the fish by reducing oxidation.

Lectins were shown to have detrimental effects on growth and insulin production at all levels tested. Trypsin inhibitors decreased trypsin production by the fish, decreased protein digestion and increased mortality at the highest levels tested. However, diets extruded using a higher processing temperature and shorter retention time in the extruder barrel were found to show better growth and feed conversion. Saponins were found not to be detrimental to salmonids and some benefits such as immune systems stimulation were demonstrated in Atlantic salmon.

Soy isoflavones (the suspected pytoestrogens) did not have an estrogenic effect on growth but did inhibit smoltification (the physiological changes needed to adapt from freshwater to salt water) of Atlantic salmon. Soy genistein does transfer to the fillets of processed fish and seem to reduce oxidation of the fats during storage.

2.4.4. Manufacture of organic fish feeds: Although allowable ingredients to gain the designation of "organic" vary depending on the certifying agency, it is generally considered that solvent-extracted soybean meal will not be allowed. Current manufacturers of organic-labeled fish feeds use toasted, expeller-pressed soybean meal. Nguyen (2008) suggests that the demand for organic aquaculture products will increase by from 5 thousand metric tons in 2000 to over 1.2 million metric tons by 2030. Nguyen (2007) has compared growth of red tilapia fed a SBM-based diet using solvent extracted SBM and expeller-pressed SBM, and found no significant difference in final mean weight, survival and feed conversion ratio.

2.4.5. Soy in diets for salmonids and marine species of fish and crustaceans: For salmonids, some progress had been made with the more concentrated forms of soy protein such as SPC. Further research is needed on the amino acid requirements of a large number of marine fish and crustaceans before appropriate diets can be formulated. Although the amino acid taurine has been identified as a key requirement and limiting factor in marine diets for some species, other species do not exhibit this need. Digestibility coefficients also need to be examined for many more species.

2.5 Conclusions

Plant protein can be the sole protein sources for fish feeds used to grow advanced fingerlings (juveniles) to market size for a variety of omnivorous freshwater species. Solvent extracted dehulled soybean meal is the main component of these feeds although other protein sources such as corn and wheat gluten meal and the various oilseeds have been used successfully. Diets for younger fish in this category, as well as for carnivorous freshwater fish, require higher levels of protein and therefore need either protein concentrates with supplemental crystalline amino acids or small amounts of fish meal to be added to a soybean meal-based diet. Low antigen feed grade SPC will become economically feasible as fish meal prices increase.

Increasing the use of soy products in diets for marine fish and salmonids, is more challenging for the freshwater omnivores. The presence of anti-nutritional factors will mean that further refined sources of soy protein will be needed. Use of soy and other vegetable oils can satisfy need for energy but not the need for n-3 polyunsaturated long chain fatty acids.

The tools at hand for overcoming the problem of sustainable inputs in aquaculture diets are: selective breeding, and genetic modifications through transgenics on the aquatic organisms and/or the feed ingredient crops (NRC, 2011).

References

AAFCO, 2006. Official Publication; AFCO Headquarters Office 1800 S. Oak Street, Suite 100, Champaign, IL 61820-6974 Email: aafco@aafco.org

American Soybean Association – International Marketing, Southeast Asia, 2005.Soy Importers Guide M04GX19403-092005-0500

Baize, J.C. 2014. A PowerPoint presentation by John Baize, consultant, John C. Baize and Associates Falls Church, VA, prepared for the USSEC, using data from the USDA.

Baize, J.C. 2000. Global soybean meal sampling and analysis activity; John C. Baize and Associates, Falls Church, VA. 22042; USB Project 0586 (August 2, 2000)

Brown P.B. and K. Smith, 2009. Soybean use-aquaculture. Sourced online at http://www.soymeal.org/FactSheets/aqua.pdf)

Cremer, M.C., Z. Enhua, Z. Jian and T. O'Keefe, 2008. Soy Protein Concentrate as a Replacement for Fishmeal in the Fingerling Diet for Common Carp, Grass Carp, Tilapia and Channel Catfish. ASA-IM/China Aquaculture Program; American Soybean Association, Beijing, P.R. China.

Cremer, M.C., Zhang, J., Zhou Enhua, 2004. Production of Red Tilapia in Ponds in Beijing with Soy-Maximized, Extruded Feed**.** Results of ASA/China 2004 Feeding Trial 35-04-96**.** American Soybean Association, Beijing, P.R. China.

Cremer, M.C., Zhang, J., Zhou Enhua, 2001. Grass carp performance on a low fat, high fiber feed formulated with soybean meal and soy hulls as the primary protein and fiber sources. American Soybean Association, Beijing, P.R. China.

Cruz, M.E., 1975. Determination of nutrient digestibility in various feedstuffs for channel catfish. Ph.D. Dissertation, Auburn University, Alabama, USA.

Davis, A.T., Stickney, R.R., 1978. Growth responses of Tilapia aurea to dietary protein quality and quantity. Trans. Am. Fish. Soc. 107, 479-483.

El-Saidy, D.M.S.D., Gaber, M.M.A., 2002. Complete replacement of fish meal by soybean meal with dietary L- lysine supplementation for Nile tilapia Oreochromis niloticus (L) fingerlings. J. World Aquaculture Soc. 33, 297-306.

Enhua, Z., Z. Jian, M.C. Cremer and T. O'Keefe, 2010. Tilapia LVHD Cage Production with 24%, 28%, 32% and 36% Protein Soy-Based Feeds. 2010 Tilapia 4 Protein Level LVHD –Hainan. American Soybean Association, Beijing, P.R. China.

Gatlin, D.M. III. 2002. Use of Soybean Meal in the Diets of Omnivorous Freshwater Fish Department of Wildlife and Fisheries Sciences, Faculty of Nutrition, Texas A&M University System; sourced at ***soymeal**. org/FactSheets/OmnivorousFwFish TechReview.pdf* AquaSoy Initiative, United Soybean Board and American Soybean Association)

Guinn, J.M., 2002. Domestic Quality Standards and Trading Rules and Recommended Export Contract Specifications for U.S. Soybeans and Products; 17 pages, online sourced at: http://ussec.org/resources/trade-rules/

Hart, S. and P.B. Brown, 2005. Soy in Aquaculture partnership studies soybean meal use in fish feeds. Article prepared for the soy-in-aquaculture Managed Research Program.

Kearl, L. C., D. LAI Dinh, F. Jager and L. E. Harris, 1980. International feed databank system, Prepared on behalf of INFIC by the International Feedstuffs Institute, Utah Agricultural Experiment Station, Utah State University, Logan, Utah 84322

Lim, C., C. D. Webster, and Cheng-Sheng Lee, 2008. Alternative Protein Sources in Aquaculture Diets, ISBN: 978-1-56022-148-7

Lim, C., Akiyama, D.M., 1992. Full-fat soybean meal utilization by fish. Asian Fisheries Science 5, 181-197.

Lim, C. and W. Domini, 1989. Utilization of plant proteins by warm water fish. In: Applewhite, T.H. (Ed), Proceedings of the World Congress on Vegetable Protein Utilization on Human Foods and Animal Feedstuffs. American Oil Chemists' Society, Champaign, Illinois, pp. 245-251.

Masuda, T. and P.D Goldsmith, 2009. World soybean production: area harvested, yield and long-term projections. International Food and Agribusiness Review 1:4 143-162

Nguyen, T.N., 2007. Total sulfur amino acid requirement and its application to practical diets for juvenile tilapia (*Oreochromis spp*.) PhD dissertation, Auburn University, Auburn, Alabama, USA.

Nguyen, T.N., 2008. The utilization of soybean products in tilapia feed-a review. In 8th International Symposium on Tilapia in Aquaculture. P53-65.

NRC, 2011. Chapter 16 Replacement of marine resources: effects of alternative ingredients and strategies on nutritional quality, *in* Nutrient Requirements of Fish and Shrimp. National Academies Press, Washington D.C.

Saad, C.R.B., 1979. Use of full-fat roasted soybeans in practical catfish diet. M.S. Thesis, Auburn University, Auburn, Alabama, USA.

USSEC, 2011. Shrimp feed trial- Venezuela. Sourced online at: http://www.soyaqua.org/sites/default/files/reports/shrimpfeedingtrial.pdf

USSEC, 2013. Aquaculture marketing brochure. Sourced online at: http://ussec.org/resources/technical-library/ as aquaculture-marketing-brochure.pdf

U.S. Soybean Export Council > Resources > Buyer's Guide, chapter 2 Quality Standards for US soybeans and soybean products.

Van Eys, J. E. Manual of quality analyses for soybean products in the feed industry, 2nd Ed. GANS Inc., 24 Av. de la Guillemote, 78112 Fourqueux, France ; jvaneys@gmail.com Sourced at : http://ussec.org/wp-content/uploads/2012/09/Manual-of-Quality-Analyses-2nd-edition.pdf

Viola, S., 1975. Experiments on nutrition of carp growing in cages part 2. Partial substitution of fish meal. Bamidgeh 27,40-48.

Wilmar International, Singapore website: http://www.wilmar international.com/library

Zarate, D.D., Lovell, R.T., 1997. Free lysine (L-lysine·HCl) is utilized for growth less efficiently than protein-bound lysine (soybean meal) in practical diets by young channel catfish (Ictalurus punctatus). Aquaculture 159, 87-100

World Bank, sourced at http://knoema.com/WBCPD2015Jan/world-bank-commodity-price-data-pink-sheet-january-2015

CHAPTER – VII

FEED TECHNOLOGY

Author
Dr. Mirza Zafar A. Khan
General Manager
Kashmir Feeds Ltd.
Lahore-Pakistan
E-mail: zafar.khan65@gmail.com

Co-Author
Engineer Dr. Ather Ibrahim, Ph.D.
Assistant Professor
Institute of Advanced Materials,
Bahauddin Zakariya University
Multan-Pakistan
E-mail: atheribrahim@yahoo.com

Editor
Dr. Carlos A. Campabadal, Ph.D.
Outreach Specialist/Instructor in
Feed Manufacturing and Grain Storage
International Grain Program Institute
Department of Grain Science and Industry
Kansas State University
Manhattan, KS, U.S.A.

INTRODUCTION

Aquaculture world-wide is an emerging agricultural sector with an average growth of more than 10% per annum. According to the FAO (2014) statistics, 154 million metric tons of fish were produced in 2012 in the world, of which 41% was produced from aquaculture. It is expected that aquaculture will produce 120 to 130 million metric tons by the year 2020 (FAO 2014). Fish consumption per capita is growing rapidly due to its importance in human nutrition and the need for more food protein sources. Recent data show that 85% of the total fish farmed or wild caught is used for human consumption and remaining 15% is used for fish meal and fish oil production. The future fish demands will be met by intensification of fish culture practices in existing operational units, by introducing more flow-through and recirculation fish culture systems, cage culture and pen culture. In sustainable intensive and semi-intensive aquaculture operations, feed costs represent up to 60 - 75% of the total operating costs. Intensification of fish culture is the most viable option to adopt for higher production and to bridge the widening gap between demand and supply. This high production oriented approach cannot be accomplished without availability of quality feed in adequate amounts.

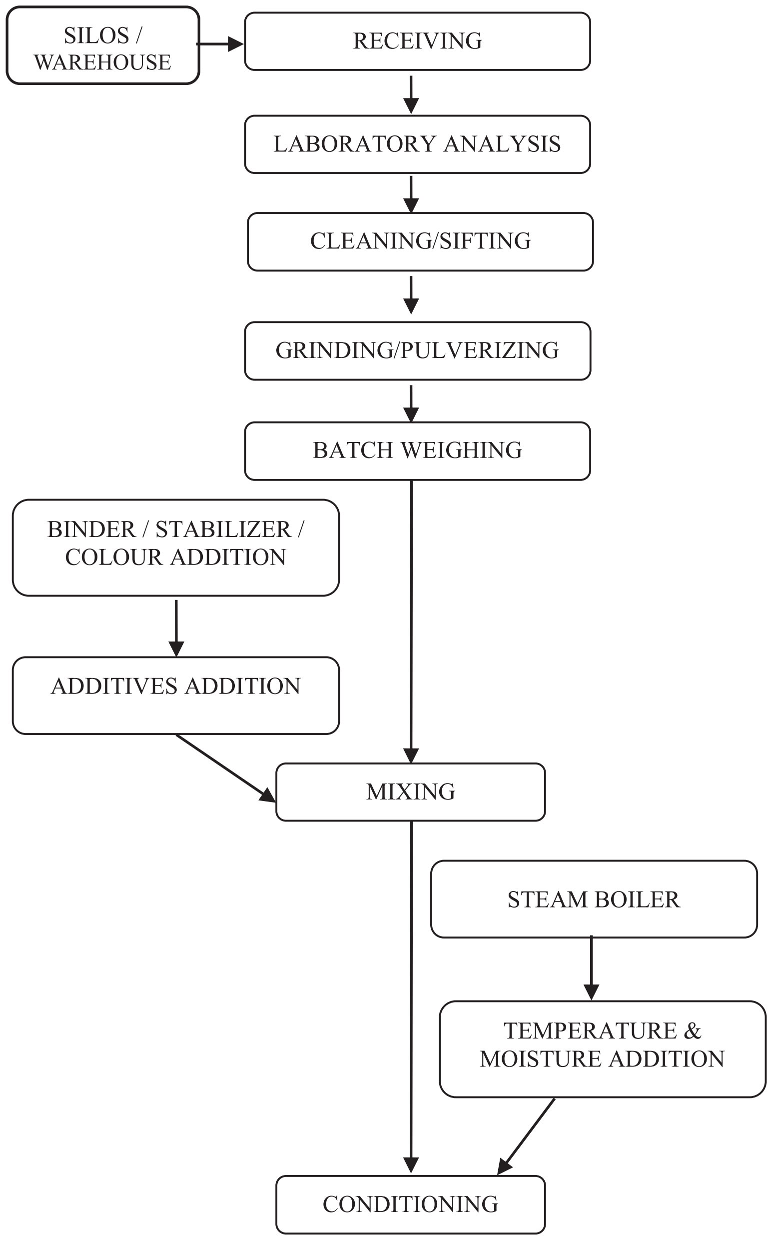
The manufacturing of aqua feeds is more complex than the common feed manufacturing for terrestrial species of livestock. Fish demand high protein content feeds in well bound forms with minimum leaching of the nutrients into the aquatic environment. Therefore both chemical and physical attributes of manufactured feed play an integral role in sustainable and commercially viable aquaculture. Feed quality promotes good growth with efficient feed conversion, help in maintaining good water quality and health of fish. Selection of quality ingredients is the foremost step for production of quality feed. Every possible measure should be adopted to ensure quality and safe delivery of the ingredients to the production unit. Prior to formulation make sure that ingredient will provide the concentration of nutrients we are expecting. It is advisable to chemically analyze all the ingredients in all the new supplies to avoid uncertainty.

The diets produced for aquaculture enterprises can be in powder, flake, mash, pellet, crumble, or extruded floating forms. The buoyancy can be directed in the processing for sinking, slow sinking or floating feeds. The physical forms of feed in aquaculture are very important and certain modifications are required in conventional production methods to manufacture feed which suits the type of species cultured and life stage. The following table shows a typical relationship of feed particle size with age and weight of a farmed fish.

Table 1. Relationship of feed particle size with fish age and weight

Fish Weight	**Crumbs Size mm**
2 to 30 days	0.5 mm
2 gram	1 mm
20 gram	1 – 2 mm
20 – 100 gram	2 – 3 mm
100 – 250 gram	3 – 4 mm
Above 300 gram	4 – 6 mm

The ingredient composition of the feed formula has an impact on the nature of feed and manufacturing process for aqua feeds. Feed types require various processing conditions and equipment to acquire different levels of temperature exposure, gelatinization and liquid addition in form of moisture and fats. Different feed ingredients like cereal grains, agro industrial by-products and animal protein sources pass through various milling and processing techniques to manufacture quality and efficient aqua feeds. The feed production process for aqua feeds generally is comprised of different processing steps (Fig. 1) to produce good quality feeds.



Flow chart continues on next page

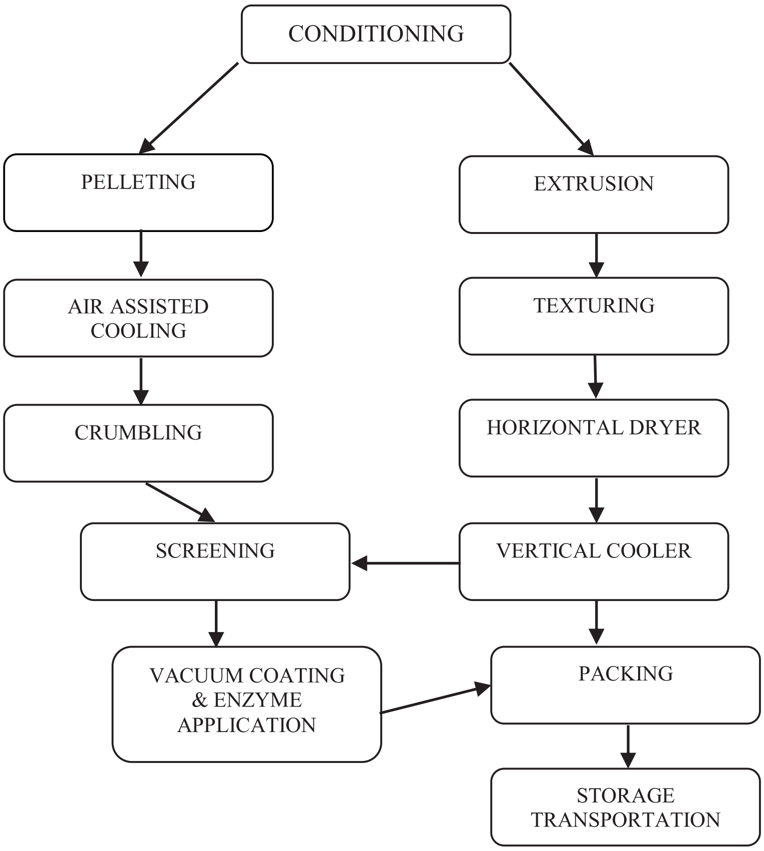


Figure 1. Flow Diagram of Aqua Feeds Manufacturing Processes.

FEED PLANT DESIGN AND SITE

The design and structure of feed plants vary considerably due to area, transportation / logistics, availability of the raw material, nearest fish markets and approaches, zoning restrictions, and type of labor available. Small modular turn-key plants can serve the new area of aquaculture development. The capacity of the plant involves selection of the processing equipment and plant design. The equipment selection is very important and sometimes the purchase cost of the equipment is insignificant in comparison to its operational cost. For instance, on-farm feed manufacturing can be operated in a simple warehouse with the manual operation of batching, mixing and processing but it is much more complicated when we move to commercial production. Commercial plants entail complex factors like capital investment and pay back periods, feasibility, comprehensive surveys, grain storage silos, warehouses, up-stream and down-stream state of the art equipment, forward contracting for bulk ingredients, inventory controls, utility connections, flexibility in producing various types of feeds, future expansion capability, quality control, skilled manpower, commercial competitiveness, value addition, branding, environment friendly, extension work, marketing, and after sales technical services. When working commercially the processing equipment has the following key segments to accomplish the pelleting process.

- Grinding
- Screening
- Mixing
- Pelleting
- Extrusion
- Drying
- Cooling
- Crumbling / Sifting
- Coating

GRINDING (Particle Size Reduction)

The grinding of the raw materials is one of the major pre-requisites in aqua feeds manufacturing. Grinding of raw materials reduces particle size and increases surface area, reduces moisture, facilitates mixing, reduces density variations and increases digestibility, increases palatability, and acceptability. Grinding should be organized to achieve requisite sized material as large particles are undesirable and even dangerous for small fish and fry. Coarse or ultra-fine grinding depends on feed type and supports pelleting or extrusion process by lowering the extra wear and tear of the equipment and consequently increasing the production output. As a general rule of thumb, material grinding level should be around 1/3 of the openings of the die. While producing feeds below 1mm size, 80 to 85% of the material should be less than 1/5th, the size of the die opening. For crumbles of less than 500 micron size the spectrum of the meal should have 80% less than 100 micron size. Particle size reduction depending on the equipment used, can involve immense energy utilization and will depend on several factors that will be mentioned below for each specific equipment. Grain type, size of sieve size, hole diameter, tip speed of the hammers, single or double pass pulverizing and degree of grinding will also have an impact.

The equipment used for particle size reduction include:

- Hammer mills
- Roller mills
- Pulverizers

HAMMER MILLS

Hammer mills accomplish size reduction by impact on a slow moving cereal grain with a fast moving hammer. Hammer mills work on the principle that most materials will crush, shatter or pulverize upon impact. The target has low kinetic energy and is struck with a heavy force of the high speed moving hammers which are attached to a shaft which rotates at high speed inside the chamber. The hammer tip travels at 15,000 to 18,000 ft. per minute. (76.2 to 91.4 m/s). The quantity, size, arrangement and speed of the hammers, screen size, distance among the screen and hammer produces coarse to fine grist spectrum as well as determines the mill capacity. The energy utilization depends upon degree of grinding. Hammer mills are less capital intensive compared to the roller mills and pulverizers but at the same time are less efficient users of energy in comparison to the other mentioned machines. The effective screen / sieve size of hammer falls among 1 to 3 mm scattered holes for aqua feeds grinding requirements.

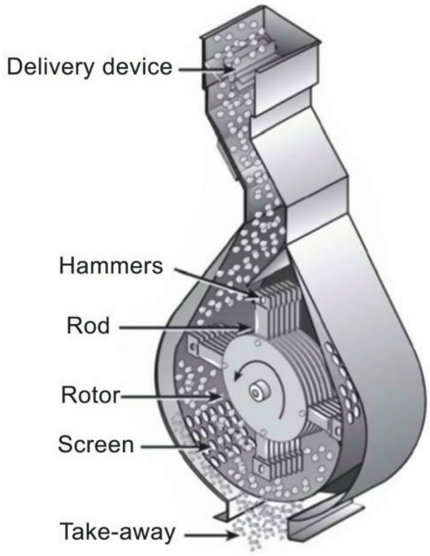


Figure 2. Internal view of a hammer mill

ROLLER MILLS

Roller mills accomplish particle size reduction through combination of cutting and crushing by corrugated rollers. The rollers moving at uniform speed create compression while rollers moving at different speeds create two way action of compression, shearing and tearing action. Roll grinding is economical and efficiently works on dry ingredients low in fat. Roller speed also matters for throughput of the grind. The typical speed ranges from 1,300 to 3,000 ft. per minute (6.6 to 15.2 m/s). Particle size can be achieved from 500 to 900 microns for cereal grains. Roller mills on an average grind size are 50% more efficient than hammer mills in terms of kilo-watt per hour of energy.

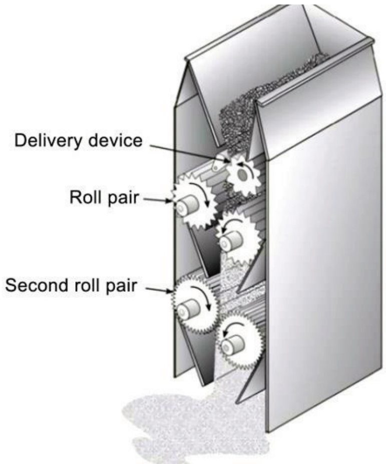


Figure 3. Grinding process is a roller mill

PULVERIZERS

Pulverizers are used for ultra-fine grinding of aquatic feed raw materials required for special processing. Pulverizers work by impacting particles with their beater plates at very high speed by moving them to corrugated liners and reduce size of the material in microns. The process of hitting continues again and again altering density reduction ultimately leaving the machine through the air stream to a product collector.

Pulverizers are used for tilapia, larval and shrimp feed production, which generally utilize 0.5 mm to 1 mm maximum in particle size. When grinding screen size of 1 mm or below is used in hammer mills, the capacity due to the screen's hole size significantly drops and may result in reduced quality, production efficiency and increases the maintenance cost of the unit.

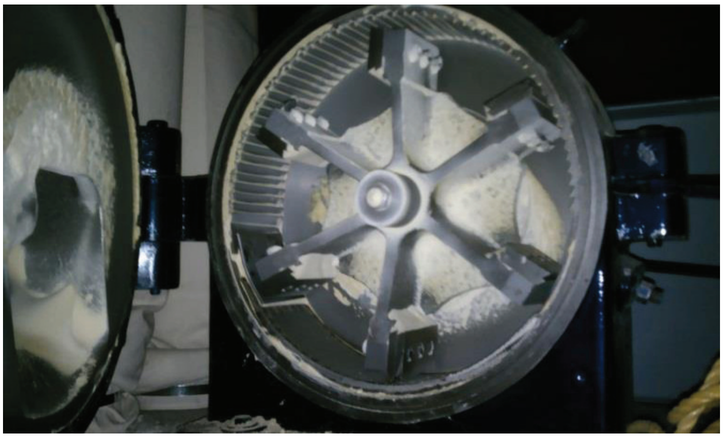


Figure 4. A typical Pulverizer for fish feed production

In this case it is more efficient to use an air classified mill (pulverizer) to get dramatically higher throughput of fine grind. Pulverizers are cost intensive at installation. Advantages of ultra-fine grinding are;

- Meets special feed and processing requirements
- Improves water stability of the feeds
- Identical density keeps nutritional balance intact
- Efficient through put grind of mixed feeds can be attained
- Reduce wear on extruder dies
- Energy efficient in comparison to other grinding systems
- Produces less heat during particle size reduction

SCREENING

The type and quantity of ingredients selected are based upon two factors; fish species and its size or age. Feeding habits are also to be considered. Some species learn quickly to eat floating feed whereas others, more used to searching for food at the bottom of the pond, may take longer to train or like shrimp, may need sinking diets. Size and age of fish will determine the type of nutrition to be added as well as the size of the feed pellet.

In order to produce quality feed economically and to ensure desired feed conversion rate as well as disease free rearing of fish, the ground raw materials must be segregated as per their particle size by passing through a sieving system either composed of multiple sieves shaken through gyratory vibratory motion or of an inclined rotary sieve bed. Correct particle size is also necessary to ensure correct proportions of all ingredients as per the feed formula in each feed unit. The particles larger than the required size or "overs" are examined and then based upon their nature and requirement are either subjected to regrinding or expelled from the production line as rejects. One undesirable result of grinding is generation of excessive very fine particles, or dust, especially when dry raw material is being processed. Although quantity of these fines can be reduced by employing various methods such as spraying of oil or by using higher moisture content raw material, it is essential that these dust particles must be segregated from the bulk through properly designed dust collectors. The collected fines can then be processed appropriately either to prepare feed for fingerling or fry fish or utilized for other purposes. These raw material dust particles may separate from the feed in water and accumulate on the gills of the fish where they can create a breeding ground for bacteria and parasites resulting in gill disease.

MIXING

Mixing is carried out either to blend or to scatter the individual components in the mix so that each unit part of the mix contains all the ingredients either in the desired proportions or very close to it. Thus proper mixing is very important from the quality control point of view. Mixing is considered more of an art than any specific science and is conducted more on experience than anything else.

Within the context of this handbook, mixing involves mixing of solids as well as liquids both separately as well as combined. In addition to type of mixer, correct mixing depends upon differences in physical properties of the various ingredients of the mix. In the case of solids; size, density, shape, coefficient of friction and electrostatic charge determines distribution of ingredients, whereas in the case of liquids; viscosity, miscibility and base play vital roles. In fact, in solids the most important factor is the size distribution of the ingredients, which may lead to randomized mixing or segregation. It has been experimentally established that small uniform sized ingredients will result in a mixture that is close to the distribution as per the formula.

Mixing can be done manually in batches either with shovels on dust protected floors or in suitable containers. It can also be done as batch or continuous processes in mechanical mixers. Generally fish feed is prepared in batches after mixing in mechanical mixers. For this purpose the large volume ingredients are added first to the mixers followed by any medium volume ingredients like salt, calcium, or premixes, then the small quantity ingredients are added and in the end the liquids are added. These mixers are made in different configurations, shapes, and sizes depending upon the buyer's requirement, such as revolving drum, straight or tapered sided cylinder, or cone shaped, horizontal or vertical, top or bottom loading, ribbon, scrapper, paddle, plough or screw mixers. Rotation speed and mixing time are very important factors in ensuring proper blending free of lumps and with minimum possible size reduction of the contents. Methods employed to verify mixing uniformity (evaluation of the degree of mixing of each proportions of the formula's ingredients) are based on the measurement of coefficient of variance (CV). This quantification utilizes water soluble tracers in the mix that are quantified using laboratory. Another important mixing process applicable to fish feed preparation is called premixing. It involves mixing of micronutrients such as vitamins and minerals. This process can also be utilized to include fish medications in the feed on required basis. Premixing is also carried out in batch type mixers for recommended preset time to ensure proper blending. A well-mixed batch of ingredients in powder form is often referred to as a "mash". In some extensive pond farming systems the mash may be broadcast by hand. Even though fish may aggressively consume the mash, many particles are lost and end up just fertilizing the pond ecosystem.

PELLETING

Pelleting is an agglomeration process that consists of changing mash feed into solids of given shape or texture by means of a mechanical process in combination with moisture, heat and pressure. The moisture and heat are inducted through saturated steam (steam at almost 100% quality) and/or mechanical pressure. The pressure obtained mechanically by pressing the feed by the rollers through the die, is around 300 to to 600 kg/cm2. In the steam addition process during the conditioning of the mash, the steam will increases the temperature and moisture of the mash. The rule of thumb is that for every 10 0C rise in temperature, it will typically increases the mash moisture by 0.7 to 1 %. Hence, the overall goal of the conditioning is to add between 4 to 6 % moisture into the mash in order to initiates gelatinization of starches in the outer layer of the particles. The pelleting processes started a century ago based on a ring die principle applying specific mechanical energy and still continues as a major processing technique in feed processing and product densification for various species of livestock and sinking aqua feeds.

After the conditioning process, the hot and moistened mash is compressed outward through cylindrical die by the rollers to produce the pellets. This process of forming the pellets, changes the mash density of 0.4 g/cc to a pellet bulk density of 0.5 to 0.6 g/cc.

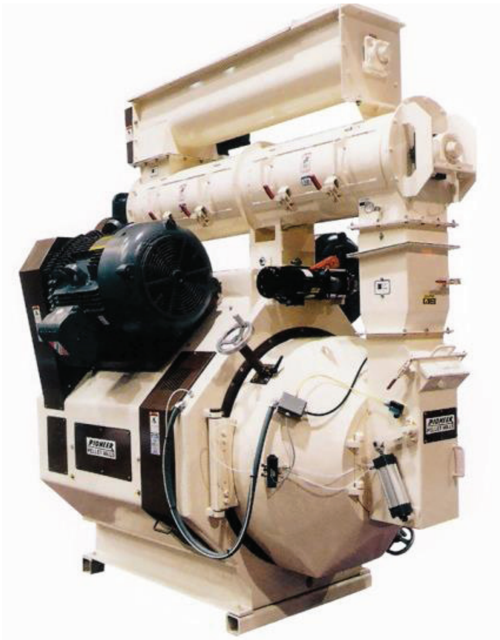


Figure 5. A compression pelleting machine

Pelleting process can be accomplished in three stages:

- Conditioning
- Compressing
- Cooling

After cooling, in some cases it is required to have a smaller pellet size, therefore, in a Post-pelletizating process called crumbling, the pellet may be broken into smaller pieces. This crumbling process assures that the proper mix of ingredients is maintained and that the crumble is "bite" sized for small mouths in younger fish. At the same time, after crumbling, a sifting step is almost always required as the breaking process inevitably produces small particulates that would otherwise become fines in the feed. The material sifted is often fed back into the original pelleting process. The many advantages that pelleting process offers over mash feeds include:

- Pellets have better flowing characteristics
- Improves bulk density of the rations
- Reduces segregation of the materials
- Reduces feed wastage
- Reduces feed degradation by controlling bacterial and fungal populations
- Pellets provide a "mouthful" of balanced feed
- Improves nutrient utilization through better digestibility
- Improves uniformity and robustness of the fish harvests
- Pellet mills are available in capacities of 1 to 35 T/h of feed production and allows flexible selection for installed capacity

Pellet mill operation is considered very sensitive due to of the characteristics of its input and output variables. The input variables can be divided into conditioning and pelleting.

INPUT VARIABLES

- Mash flow(conditioning)
- Steam condition and pressure (conditioning)
- Particle sizes of the mash (conditioning and pelleting)
- Distance between die and roller shell (pelleting)

OUTPUT VARIABLES

- Size of the pellet
- Pellet durability
- Pellet moisture content
- Pellet temperature.

Pellet durability and hardness are quality factors that are considered objective, while color, texture, dust level and palatability are subjective.

PELLET MILL DIE SPECIFICATIONS

- Entry taper of the hole
- Size of the hole
- Depth of the taper
- Length of the hole
- Angle of the taper
- Angle and depth of the relief taper

CONDITIONING, PELLETING PROCESS AND COOLING DISTURBANCE VARIABLES

- Properties of the mash
- Moisture of the mash
- Temperature of the mash
- Relative humidity
- Ambient air temperature.
- Steam type, saturated, dry, over heated
- Velocity of the steam
- Roller shell, die type and condition

EXTRUDERS

Extrusion can be defined as a technological process of forcing feed raw material in one or more of the processing conditions (such as mixing, heating, cutting, etc.) by making some chemical and physical changes through the die to make material forming or eruption gasification. Extrusion allows buoyancy control to make floating, sinking, or slow sinking feeds. The bulk density of the extruded aqua feeds can be reduced by 0.25 – 0.3 g/cc compared with mash prior to extrusion. The extrusion process may or may not involve a simultaneous cooking process and this process functions in many ways including texture alteration, grinding, hydration, expansion, homogenization, mixing, partial dehydration, shearing, protein denaturing, gelatinization and shaping. A properly conditioned mash that has substantial levels of water and temperature in a range of 100 to 1600C, will gelatinizes the starches and make proteins glutinous to increase the dough viscosity. After the mash is conditioned and cooked throughout the extruder, the mash can then be mechanically converted into expanded floating or sinking aquatic feeds.

The extrusion cooking for aqua feeds may involve using high temperature steam in the extruder barrel for 5 to 10 seconds in order to raise the mash to 2000C. Some of the benefits that are obtained are:

- Improved digestibility
- Improved feed conversion ratio
- Inactivates certain anti–nutritional factors
- Kills gram negative and aerobic bacteria
- Controls mold and yeast growth and destroys lipases
- Enhances energy liberation through breaking starch and fat molecules
- Enhances amino acids availability
- Improves palatability and
- Improves water stability of feeds up to 12 hours

Extruders have the characteristic that they can handle mash feed with moisture content between 20 and 40%. The extrusion mechanism depends on certain independent variables and dependent variables. Independent variables are further divided into two parts of conditioning and extrusion.

Figure 6. E-750 extruder machine with accessories.

The conditioning variables include critical parameters like temperature, moisture content, and retention time. In this phase the product responds with certain chemical and physical changes. While in extrusion phase the most important variables are: moisture content, oil addition, thermal energy, mechanical energy application, and retention time. The dependent variables include feed rate, lighter or heavier densities, and extent of cooking which can determine between the sinking and floating feeds because less cooking will lead to less expansion, high density and sinking feeds. High expansion on the other hand will result in low density floating feeds. The starch contents of the rations will also direct the ultimate feed type production. Starch is the primary form of carbohydrates found in aquatic feeds and levels like 10% starch tend to form sinking feeds while, 20% starch based formulations tend to turn into floating feeds. Amylose based starches have better binding and gelatinizing ability and convert into more water stable aqua feeds. The water activity is also an important factor in the shelf-life of extruded feeds. The ratio between vapor pressure of the water above the test sample and pure water or partial pressure of the water at the same temperature should not exceed the level of 0.7, since it depicts the measurement of energy status of water in a system and at the same time, practically all the microbial activity stops at this point not allowing the growth of contaminants.

CONDITIONING

Conditioning refers to the preparation of the mash with the aid of steam added heat and moisture before it is compressed by the extruder mechanical force and pressure. Conditioning has a great bearing on compression process, palatability, durability and thus on quality of the extruded feeds. Proper conditioning of the mash before extrusion largely depends on the grist spectrum of the particles and generally the spectrum described below (Table 2) is considered suitable for efficient operation.

Table 2: Relationship between particle size and the maximum amount in a fish feed formula

Size Of The Particles.	% Level In The Meal.
3.5 mm	Up to 1 %
2 mm	Up to 5 %
1 mm	20 %
500 micron	30 %
250 micron	25 %
< 250 micron	Not less than 20 %

The purpose of the conditioning can be highlighted as,

- Equalizes moisture level of different raw materials
- Reduces wear and resistance during compression
- Reduces bacterial / fungal count
- Enhances adhesive properties
- Builds up bridges of solids

The conditioning process creates certain modifications for enhancing nutrient availability to the fish. It improves gelatinization of the starches, makes room for high fat and liquid addition, improves pellet quality, and intestinal digestibility.

Figure 9. A pre-conditioner with feed hopper

Crude fiber modification liberates more energy and creates more flexibility in using fibrous ingredients. Protein modification enhances digestibility through denaturizing process, reduces amino acids wastage as in high temperature steam treatment, reduces nitrogen excretion, alleviates restrictions in the use of synthetic amino acids, and plasticizes/coagulates proteins. The conditioning process kills most gram negative bacteria, molds, aerobic bacteria and improves feed hygiene.

Steam quality also affects the conditioning process such that the steam entering the conditioner should not just add temperature but should also add moisture. The pressure of the steam through the feeding lines should be between 5 to 7 bar, but when it enters the conditioner, its pressure should be reduced through a PRV (Pressure reducing valve) to 1 – 1.5 bar. At the same time, the velocity of the steam is also altered by increasing the pipe diameter by 2.5 times just after the PRV to a recommended velocity is 20 m/s. Generally, the ideal quality of the steam should be as close to 100% as possible, therefore, as saturated steam. The conditioning process may be short or long term by means of retention time and type of the conditioner. Twenty seconds to 1 minute time is considered as short term conditioning while dwell time for long term conditioning can be 3 to 10 minutes increasing ripening of the mash and enhancing gelatinization prior to the extrusion process.

Different types of conditioners are used for varying temperature and moisture content levels of the mash prior to extrusion.

- Small barrel conditioner mixer with paddles
- Two stage barrel conditioner with paddles.
- Long conditioner LLX type
- Differential diameter cylinder with varying speed in each chamber

The differential diameter cylinder conditioner has more popularity for conditioning aqua feeds. It improves average retention time/distribution in the conditioner. The shaft speed also affects mixing as the small chamber shaft operates on high RPM in comparison to the large side which works on a lower RPM. It offers more flexibility for adding more water and oil during conditioning. Steam in the conditioner is added from the bottom while other liquids are added from the top. In terms of filling of the conditioner, 40% filled gives more homogenized ripening and gelatinization of the meal prepared for extrusion.

Gelatinization is described as the swelling of starches with heat and moisture. Starch granules are generally insoluble in water below 500C. When a suspension of starches in water is heated beyond critical temperature, the granules absorb water and swell to many times of their original size. The suitable temperature for this purpose will be 60 to 800C. The swelling of starches due to colloidal dispersion makes starch paste defining gelatinization. True gelatinization of starch molecules occurs when a paste is cooked at 100 to 1600 C (HTST) which is usually required for mash having 20% and above starch content for floating aqua feeds.

FLOATING FEEDS

To understand the floating characteristics of the pellets, its density should be below 1g/cc in comparison to water density in order to float. If the pellet has a density of 1.05g/cc in freshwater, and 1.12 g/cc at 3 % sea water, it will sink. Therefore, in order to make the pellets float, certain processing parameters should be reached. Following is an example of operating parameters that can be used to produce floating feed in an extruder. The conditioning time may range from 120 to 140 seconds and the mash should have a temperature between 95 to 1100C with a moisture content between 22 to 26%. The temperature of the mash at the discharge end of the extruder should be 1300 C, moisture content between 21 to 24%, and a pressure at the discharge between 34 to 37 atm. The bulk density should be less than 480 and 440 g/l in fresh water and sea water, respectively.

Figure 7. Floating feeds of various shapes and sizes

SINKING FEEDS

For sinking feeds, the operating parameters of the extruder are different when compare to producing floating feed. For example, the conditioning time may range from 90 to 120 seconds and the mash should have a temperature between 85 to 950C, and a moisture content between 25 to 32%. The temperature of the mash at the discharge end of the extruder should be at 1200C, the moisture content of the mash between 24 to 27%, and the pressure at discharge between 20 to 29 atm. The bulk density of mash to be extruded into pellets should be less than 600 and 640 g/l in freshwater and sea water, respectively.

Generally, there are two types of extruders that are used for manufacturing aqua feeds:

- Single screw extruder
- Double screw extruder

Also, the extrusion process may be dry or wet cooking depending on the addition of water in the conditioning process.

Single screw extruders have three types:

- Dry cooking extruders
- Wet cooking extruders
- Cold forming extruders

Generally dry cooking extruders are operated without steam and are used in processing of low moisture highly expanded starch products. These are also used for processing whole soybean to full fat soybean meal or flour and are not commonly used in the production of aqua feeds. Due to dry cooking, the post extrusion step of drying can be eliminated. These extruders are not cost intensive and are sometimes used for small operations at the farms.

Wet cooking extruders are equipped with pre-conditioners and are largely used in the aqua feed and pet food industry. These extruders can handle 20 to 30% moisture mashes with the high capacity of production from 1 to 20 T/h with variety of raw materials. Wet extrusion cookers can handle direct water and fat injection and require a post extrusion drying and cooling process. Wet cooking extrusion improves digestibility and eliminates most pathogens. Cold forming extruders have a very limited use in producing moist aquatic feeds. These function like a meat grinder with the ability of blending certain raw materials. This extrusion does not benefit like pasteurization.

Twin screw cooker extruders are used in wet extrusion and are operated for specialized aquatic feeds manufacturing. Initial capital investment, operational and maintenance costs are higher in comparison to single screw extruders. Twin screw extruder of identical capacity as a single screw extruder can have a purchasing price 1.5 to 2 times higher and at the same time, the same factor applies in maintenance costs. Twin screw extruders can handle water and fats injection during the course of cooking extrusion and can use very small dies of 0.7 to 1.5 mm in diameter. They also offer the advantage of a resulting more clean equipment at end of the operation. They can handle formulations having 30 to 40% meat based ingredients. Overall, these extruders offer great flexibility and produce extruded products with even size and uniformity.

Extrusion cooking is not only cost intensive regarding the initial capital investment but is also expensive in operation due to the high electrical energy input requirement. For instance, small cooking extruders can use up to 0.5 kWh in producing just 1 kg of the aquatic feeds. Special emphasis should be given in selection for proper capacity installation of the equipment.

Figure 8a. An assembly of twin screws.

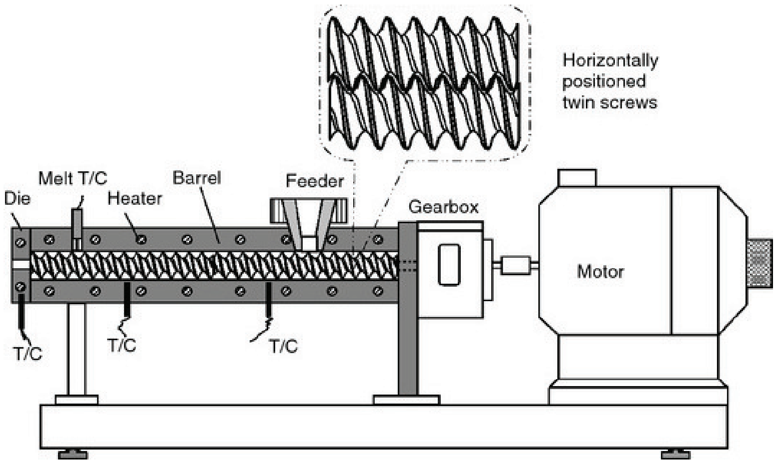


Figure 8b. An assembly of twin screws and their arrangement in the extruder.

DRYERS

The drying process in aqua feed production is more relevant in case of feeds manufactured through extrusion since their moisture contents are relatively higher. Drying with heated dry air is used for removal of excess moisture thereby increasing the shelf life of the feed. Generally, horizontal dryers equipped with single or double stage perforated conveyor belt or trays allowing flow of hot air through the thin layer of properly spread feed are employed for this purpose. Single stage horizontal dryers are a better option because it results in generation of fewer fines but they require more floor space and better control on processing parameters. Whereas double stage dryers are compact and efficient in controlling the moisture content, mechanical handling results in more fines which then have to be reprocessed to control production cost. Air temperature, speed of movement of feed through the dryer are determined by the moisture content of the feed entering the dryer and the desired moisture level of the feed leaving the dryer.

COOLERS

Coolers extract heat and surplus moisture created during extrusion/pelleting making feed harder and suitable for handling and storage. The pellets leaving the cooler should be 5 to 80C above the ambient temperature and moisture uptake by finished feed should be around 0.5 to 1% as compared to the mash compounded for conditioning. Generally, depending on their design, coolers can take 10 minutes for cooling after extrusion process. Cooling mechanism depends upon the ambient air temperature coming in intimate contact with outer surface of the pellets. The temperature imparted to the pellets by steam is a major factor in subsequent drying. The ambient air blown through the newly formed hot pellets will absorb heat and thus increases its water holding ability leading to drying initiation. The following types of coolers are used in aqua feeds manufacturing:

1. Vertical cooler
2. Horizontal belt cooler
3. Counter flow cooler

VERTICAL COOLER

In a vertical cooler the free flowing pellets by gravity makes a bed in the cooler at the full level to attain its maximum efficiency and discharge is governed by the feeding rate. Cooling is achieved by drawing ambient air cross wise through the column of pellets and exhausts through a cyclone leaving the fines for recycling.

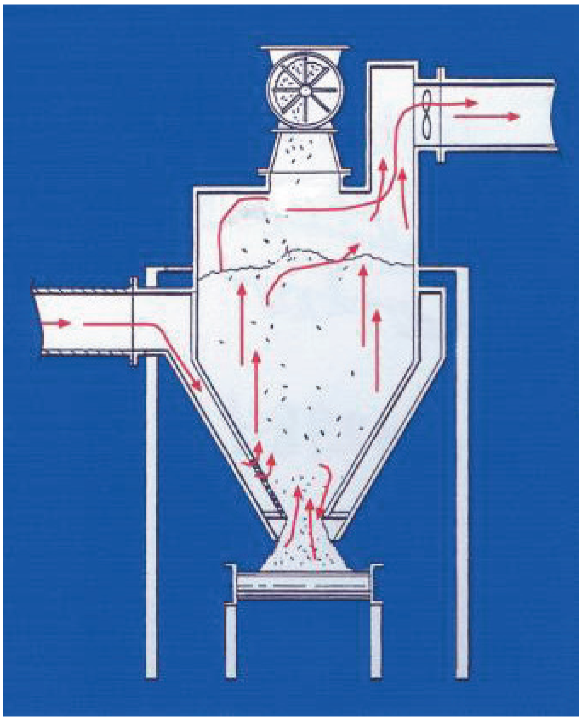


Figure 10. Air flow in a vertical cooler

HORIZONTAL BELT COOLER

Horizontal coolers are more flexible for cooling/drying extruded feeds which usually contain more moisture by controlling retention

Figure 11. A horizontal belt cooler

time of feeds. Bed depth and air flow can be controlled and better efficiency of the cooling can be achieved by completely covering the cooler bed with an even depth of the pellets to allow the air to pass through for uniform dispersal and uptake of moisture from the feeds.

COUNTER FLOW COOLERS

In a counter flow cooler the pellets and cooling air flow in opposite directions. The coolest pellets contact the cool air and the hottest one contact with the warmest air. These coolers are becoming more popular in the feed industry. The counter flow cooler provides constant retention and an even flow over the entire discharge area. These coolers are energy efficient and cover less space for installation due to vertical orientation.

Figure 12. A view of a counter flow cooler

CRUMBLING AND SIFTING

In order to reduce their size, pellets can be passed through a crumbler after cooling. This is done for obtaining a smaller feed unit more suitable according to age and type of small fish. The crumbling process provides flexibility of producing special feeds with selected granulations, eases product flow in difficult feeding systems, higher production efficiency in comparison to small pellet manufacturing using more electrical energy, and allows more economic formulations. Crumbling mechanism occurs through cutting by rollers having different flutings and running on speed differentials. The cutting of the pellets is flexible by adjusting the roller gap.

The crumbs are transferred to reciprocal multi-deck screen operation or rotary type screen operation for separation of desired crumbs for packing and removing the fines for recycling of pellets. Removal of fines is critical both to maintain economic efficiency of reusing the material, but also as fines can end up suspended in the water column thereby not only wasting the nutrients but also contributing to poor quality of water. Fines can also result s from rough handling of feed after it leaves the feed plant. In transport to, or on the farm, throwing bags of feed, piling additional heavy materials on top or walking on full bags, will grind the pellets together and produce fines.

COATING

The pellets manufactured through pellet machine or extruder are exposed to high temperatures of conditioning and compression leading to loss of potency of vitamins and enzymes. Feeds normally contain 5 to 10% fat and to achieve higher levels, in some cases a coating is necessary with oils using low heat. In the same way, certain heat sensitive vitamins and enzymes (thermo-labile products) that would not normally withstand the conditioning and extrusion temperatures can also be added later during the coating process.

QUALITY CONTROL AND PRODUCT ANALYSIS

Aqua feeds production and farming in Pakistan is in its infancy compared to higher terrestrial animals. The sector being in a development stage will further define its parameters as industry expansion begins and commercial capitalization continues. Seafood preferences in the urban population and high income groups, provides hope for rapid development of industrial and quality norms. Pakistani feed act compliance and follow up of instructions pertaining to restricted medicines, heavy metals and prohibited feed additives. Further labeling will improve the quality and robustness of the aqua culture feed industry. New feed requirements in general, notably those related to traceability (of ingredients used within aqua feeds), higher degree of compliance with legislation, package recycling, carbon foot-prints, sanitation programs, environmental effects of feeds and factory effluents/ smoke, quality assurance, after sales close cooperation with fish farmers, R&D cooperation development in local area farming, and analytical state of the art laboratory testing facilities will serve as milestones in quality operation of aqua feeds manufacturing.

QUALITY ASSURANCE AND LABORATORY ANALYSES

For future export orientation the following certification programs will be applicable.

- Hazard Analyses at Critical Control Points (HACCP)
- International Standards Organization (ISO) Certifications
- Quality and Safety System for Specialty Feed Ingredients and their Mixtures (FAMI QS)
- Good Manufacturing Practices (GMP's)

The quality control programs in feed mills pertaining to ingredients, in-process, and finished products quality control that will rely on a sophisticated laboratory. The laboratory testing will further elaborate the measuring of specific components of feed ingredient sample to assure its quality. It may be chemical, physical or electronic measurement to determine quality of a product to compare with the predetermined or established standard. The testing data may be used to:

i. Evaluate ingredients and sources
ii. Nutrient evaluation of formulation
iii. Establishing product standard deviation variation and trend lines
iv. Assist in troubleshooting
v. Assures that feed meets the label guarantee
vi. Incorporates psychological satisfaction

These procedures can be accomplished either through the in-house laboratory or from any other laboratory of commercial repute. The tests for aqua feeds include; moisture content, particle size, bulk density, floatability, starch gelatinization, fat absorption, pellet durability, pellet hardness, water stability, and flow index. For these tests to be successful, a proper sampling procedure should be developed and followed for ingredient and finished feed. At the same time, it is important to establish a traceability procedure that starts at the ingredient receiving step and goes to the finished feed storage lot before it is sold or consumed at the farm. There are several testing equipment procedures that in some case utilizing high capacity sensors to quantify certain quality parameters. Among these equipment and procedures are:

i. Labeling, batching for traceability.
ii. Feed microscopy
iii. Wet chemistry application
iv. Chromatography
v. Spectrophotometry
vi. NIRS details reflectance spectroscopy
vii. Radio frequency
viii. Micro wave technology
ix. Capacitance and conductivity
x. DSC - Differential scanning calorimeter

Wet chemistry and NIRS are bench top technologies utilized in the scrutiny of the aqua feeds. The short wet chemistry procedures are included for the proximate analysis in the in-house laboratory. The following table (Reference) shows the equipment and procedure needed to quantify each of the main chemical compositions and its quality parameters present in the ingredients or finished feed:

Table 3: Equipment and procedure utilized for ingredient/feed chemical composition (AOAC Methods, 2012)

S. #	Test	Equipment(s)	Solutions required
1	Moisture/Dry matter	Silica dish, Oven, Electronic Weighing scale, Desiccators	NIL
2	Crude Protein	Weighing scale, Heater, Digestion flask, Volumetric flask, kjeldhal flask and apparatus. 100 ml beaker, Pipette, Hot plate	Concentrated Sulphuric acid, Sodium Hydroxide, 2% Boric acid, 0.1N HCl,$CuSO_4$, $FeSO_4$, K_2SO_4
3	Ether Extract	Weighing scale, Soxhlet apparatus, petri dish, Oven, dessicator, Water bath, Thimble	Diethyl Ether
4	Ash	Weighing scale, Crucible, Heater, Furnace, Desiccators	NIL
5	Moisture	Petri dish, Weighing scale, Desiccator, Oven	NIL
6	Acid Insoluble Ash (AIA)	Crucible, Hot plate, Whatman filter paper (#44), Heater, Furnace, Weighing scale	25 ml HCL (10%)

7	Crude Fiber	Weighing scale, 250ml Beaker, Hot plate, Oven, Muffle furnace, Water bottle , stirrer, Filter paper or cloth, Desiccators	1.25% H_2SO_4, 1.25% NaOH
8	True Protein	Weighing scale, Oven, Filter paper(#44), Digestion tube, kjeldhal apparatus	25 ml TCA (5%), Digestion mixture + 10 ml Sulphuric acid (Commercial), Sodium Hydroxide, 2% Boric acid, 0.1N HCl, $CuSO_4$, $FeSO_4$, K_2SO_4
9	Salt	Weighing scale, 500ml Conical flask, filter paper(#1), beaker, Shaker, Pipette 10ml, 1ml	Potassium Chromate (0.5%), $AgNO_3$ solution (0.1N) $ZnCH_3COOH$, Potassium Ferrocynide
10	Free Fatty Acid	Beaker, Hot plate, 10ml pipette, Magnetic Stirrer	Phenolphthalein, 0.1N NaOH, Ethanol
11	Peroxide Value	500 ml Beakers, funnel, Conical flask, Oven, Weighing Scale	Acetic Acid, Chloroform, Potassium iodide, Distilled water, Starch
12	KOH Solubility	Conical flask, Weighing scale, Digestion flask, Centrifuge machine & tubes, kjeldhal apparatus, Pipette, Mechanical shaker	KOH (0.2%), Digestion mixture, Commercial Sulphuric acid, (2%) Boric acid, 0.05 N Sulphuric acid

13	Aflatoxin Test	Weight scale, Blender jug, Graduated cylinder, Fluted filter paper, Microfiber filter paper, column, Aflatoxin apparatus, corvette, Auto pipette, vicam	NaCl, Methanol (HPLC grade), Bromine,
14	Mixing Test	Mechanical shaker, whatman filter paper#595, spectrophotometer	Ethanol, Methyl violet
15	Stability in water test	Screen Trays, Drying oven, Desiccators, balance	Nil

In the following section, all the quality parameters of each of the chemical composition are explained and detailed.

PROXIMATE ANALYSIS

Proximate analysis is performed for the determination of the major characteristics of feed to confirm that it is within its normal compositional parameters. This method partitioned nutrient characteristics of feed into several components: moisture content, ash (or acid insoluble ash), nitrogen free extract (or crude protein), crude fiber and crude fat.

MOISTURE CONTENT DETERMINATION

Moisture content is determined by the loss in weight that occurs when a sample is derived to a constant weight in an oven. About 20 grams of the feed sample is weighed into a silica dish previously dried and weighed. The sample is then dried in an oven for 1100C for 2 hours, cooled in a desiccator and weighed. The drying and weighing continues until a constant weight is achieved.

Calculation:

% Moisture Content = (wt. of sample + Petri dish before drying – wt. of sample + Petri dish after drying)/sample wt. × 100

DRY MATTER

Since the water content of feed varies widely, ingredients and feed are usually compared for their nutrient content on moisture free or dry matter (DM) basis.

Calculation:

% of DM= 100 - % Moisture Content

CRUDE PROTEIN

Crude protein is determined by measuring the nitrogen content of the feed and multiplying it by a factor of 6.25. This factor is based on the fact that most protein contains 16% nitrogen. Crude protein is determined by the Kjeldhal or Nitrogen Combustion method. The method involves: digestion, distillation and titration.

Digestion (AOAC 2012)

Weigh about 2 gram of the sample; add 25 ml of concentrated sulfuric acid and 5 gram of mineral mixture into digestion tube. Apply heat to digest it for 45 minutes until the digest becomes clear pale green. Leave until completely cool and rapidly add 100 ml of distilled water. Rinse the digestion flask 2-3 times.

Distillation (AOAC 2012)

Provide steam for the distillation apparatus and add about 10 ml of the digest into the apparatus via funnel and allow it to boil. Add 10 ml of NaOH from a measuring cylinder so that ammonia is not lost. Distill into 50 ml of 2% boric acid containing screened methyl red indicator.

Titration (AOAC 2012)

The alkaline ammonium borate (NH_4BO_3) formed is titrated directly with 0.1 N HCl. The titer value which is the volume of acid used is recorded. The volume of acid used, is fitted into the formula which becomes

Calculation:

% of Nitrogen = $0.0014 \times 100 \times 250 \times 6.25 / 10 \times 2$

% of crude protein = % of Nitrogen × Titration reading value

ETHER EXTRACT

The ether extract of a feed represents the fat and oil in the feed. Soxhlet apparatus is the equipment used for the determination of ether extract. It consists of these major components.

Extractor: Comprises a thimble for holding a sample.

Condenser: For cooling and condensing the ether vapors.

Procedure (AOAC 2012)

About 150 ml anhydrous diethyl ether (petroleum ether) having boiling point of 40-60°C is placed in the flask. 2-5 gram of sample is weighed into thimble. As the heated ether vapors reaches the condenser through the side arm of extractor, the extraction continues for at least 4 hours and most of the solvent is distilled from the flask into the extractor. The flask is then disconnected and placed in an oven at 65°C for 2-4 hours, cooled in a desiccator and weighed.

Calculation:

% of ether extract = (wt. of Petri dish + extract – tare wt. of Petri dish)/ sample wt. × 100

ASH

Ash is the inorganic residue obtained by burning of the organic matter of feed stuff at 400-650°C in a muffle furnace for 4 hours. Five grams of the sample is weighed into a preheated crucible. The crucible is placed into muffle furnace at 400-650°C for 2-4 hours or until whitish grey ash is obtained. The crucible is then placed in the desiccator and weighed. (AOAC 2012)

Calculation:

% of ash = (wt. of crucible + ash – tare wt. of crucible) / sample wt. × 100

ACID INSOLUBLE ASH (AIA)

After estimation of ash, add 25 ml HCL (10%) in crucible. Heat it on a hot plate for 2-3 min. Filter through the Whatman filter paper #44. Burn it on heater and put it in furnace for 2 hours. Weigh the acid insoluble ash (AIA) with crucible (W2) (AOAC 2012).

Calculation:

% of AIA = W2-W1/ 5× 100

W1 (tare wt. of crucible)

W2 (Crucible wt. + AIA)

CRUDE FIBRE

The fat free sample is transferred into a flask/beaker and 200 ml of 1.25% H_2SO_4 is added. The solution is gently boiled for 30 minutes maintaining a constant volume of liquid by the addition of hot water. The residue is then washed several times with boiling water and transferred into a beaker. Then add 200 ml of 1.25 % NaOH and boil for another 30 minutes and put the sample in a crucible. The residue is dried at 110°C in an oven for 2-3 hours and weighed. The residue is placed in a muffle furnace at 400-650°C for 2 hours. Then cooled in a desiccator and weighed.

Calculation:

% of crude fibre = (dry wt. of residue before ash – wt. of residue after ash)/ sample wt. × 100

NITROGEN FREE EXTRACT

NFE is determined by mathematical calculation. It is obtained by subtracting the sum of percentages of all nutrients already determined from 100 (AOAC 2012).

Calculation:

% of NFE = 100 – (% of moisture + % of crude fibre + % of crude protein + % of ether extract + % of ash)

NFE represents soluble carbohydrates and other digestible and easily utilizable non-nitrogenous substances in feed.

TRUE PROTEIN (AOAC 2012)

Take 1 gm sample and add to it 25 ml TCA (5%). Place it on magnetic stirrer for 15 minutes. Then shift it into the oven for 15 minutes with occasional shaking. Filter it with simple filter paper and wash it with distilled water. After filtration, transfer the filter paper into digestion tube. Now add 5 gram digestion mixture + 30 ml sulfuric acid (commercial). Next procedure is same as crude protein.

SALT

Weigh 1 gm well ground sample and put it in a ceramic dish. Heat the ceramic dish until the sample is free from smoke. Cool it at room temperature and crush it with glass rod. Add ¾ of crucible volume with distilled water. Heat it on hot plate. Filter it in conical flask with the help of Whatman filter paper no. 1. Wash 2 to 3 times with distilled water. Take 10 ml of filtrate in 100 ml beaker. Add 2-3 drops Potassium Chromate (K_2CrO_4) as indicator (0.5%). Titrate with silver nitrate ($AgNO_3$) solution (0.1) N. End point – yellow to brick red.

Calculation:

% of Salt = (Burette reading × factor of $AgNO_3$) × 100

Factor of $AgNO_3$ = 2.33

FREE FATTY ACID (AOAC 2012)

Take 10 gram sample of oil in 250 ml beaker. Take 50ml of ethanol. Boil it then add to it few drops of 1% phenolphthalein and change color with 0.1N NaOH. Color will be permanent light purple. Add it to sample and mix vigorously. Titrate it against 0.1N NaOH to bring light pink color, should be persisting for 30 Sec.

Calculation:

R × N × 282 × 100 / 1000 × 10 (SAMPLE WEIGHT)

(Should be <1)

R (reading) of 0.1N NaOH

While 282 is oil factor

PEROXIDE VALUE

Take 5 gm oil sample and add to it 30ml Acetic Acid: Chloroform (3:2). Add 0.5 ml saturated potassium iodide (KI) and shake vigorously for exactly 1 minute. Add 30 ml distilled water and titrate it against 0.1 N sodium thiosulfate ($Na_2S_2O_3$) (AOAC 2012).

Calculation:

R × N × 1000/ 5(sample weight)

KOH SOLUBILITY

Weigh 1.5 gm fine ground sieved sample in a beaker. Add 75 ml KOH (0.2%) and stir it for 15 minutes. After 15 minutes, take this sample in plastic or glass tube and centrifuge it for 15 minutes. When centrifuge is completed, take 5ml supernatant + 5 gm digestion mixture in digestion tube and add 30 ml commercial sulfuric acid in digestion tube. Digest for 60-90 minutes. Allow it to cool for 30 minutes and add 10-15 ml distilled water. Now take 10 ml (2%) boric acid solution in a 100 ml beaker and place it on distillation unit. Insert both pipes (NaOH and Distilled Water pipe) into its solutions. Turn on alkali switch, add suitable amount of NaOH (Until distillate becomes black) and turn it off. Ensure the water is always running. Turn on steam switch. Allow distillation of boric acid solution 30-40 ml. (takes 4-5 minutes). Titrate the distillate against 0.5 N sulphuric acid, color changes from green to light purple, read the end point (AOAC 2012).

Calculation:

% of KOH = (burette Reading) × 4375 / CP of this sample

AFLATOXIN TEST

Take 50 gm sample + 5 gm salt (NaCl) + 100 ml (80 ml methanol: 20 ml H_2O) HPLC grade in blender jug and shake it for 1 minute and filter. Take 10 ml filtrate sample in 50 ml cylinder and add 40 ml distilled water to make volume 50 ml. Filter the filtrated sample again with micro fiber filter paper. Now take the column, cut its tip and adjust it on stand. (Also adjust column and syringe on column). Now take 10ml filtrate sample and pass through column and 10 ml distilled water two times and then 1ml pure methanol (HPLC grade) also passes through the column. Collect this methanol in cuvette. Now add 1ml developer in this cuvette, shake it and put it in the Aflatoxin Test Kit from VICAM, wait for 60 sec and take reading (AOAC 2012). For labs that do not have an HPLC, there are less accurate strip tests that will demonstrate a positive of negative presence to a threshold level.

MIXING TEST (on Methyl Violet basis) (AOAC 2012)

Use set of free samples. Take 10 gm of feed sample each + 30 ml ethanol; shake them for 30 minutes on mechanical shaker. Then filter them with Whatman filter paper no. 595. Now take the filtrates reading on spectrophotometer: (495 wavelength). Calculate the results through computer (mean, standard Deviation and percentage CV)

PHOSPHORUS (AOAC 2012)

Take 0.5gm sample in 100ml conical flask. Add 10ml Nitric acid to it. Heat the flask on hot plate until only a few drops are remaining. Cool the flask and add 5ml per-choleric acid. Then again heat this flask on hot plate until only a few drops are remaining. Cool the flask and make volume to 100 ml with distilled water in volumetric flask. Filter it with Whatman filter paper No.1. Take 1 gm sample in the test tube and add 9ml D.W. Take 1ml diluted sample in test tube and add 1 ml ammonium molybdate + 0.4ml ANSA+ 7.6 ml D.W. Take 1ml diluted standard in the test tube + 1 ml ammonium molybdate + 0.4ml ANSA+ 7.6 ml D.W.

Blank sample:

1ml ammonium molybdate +0.4ml ANSA+8.6 ml D.W.

After 8 minutes stir the samples and take reading on spectrophotometer (720 wavelength).

Calculation:

Standard reading- Blank = A

Sample reading – Blank = B

% of phosphorus = B/A × 16

Stability in Water Test

Ten grams of uniform sized pellets are spread on a screen tray of 1002 cm surface area. Floating feed pellets may require a screened top cover to keep them in place. The screen tray holes should be smaller than the pellet size. Two or more set of samples are immersed into still water tank for 10 minutes. After the set time the tray is raised and allowed to drain. The sample is then dried in a drying furnace at 130oC for 2 hours followed by cooling in a dessicator. The sample is again weighed and its ratio to initial weight (10 grams) is used as comparative measure of stability of pellet in water.

References

AOAC Methods (2012). Official Methods of Analysis of AOAC INTERNATIONAL, 19th Edition.

CHAPTER – VIII

FISH DISEASES AND HEALTH MANAGEMENT

Author
Dr. Zafar Iqbal, Ph.D.
Professor
Fish Disease and Health Management Laboratory
Department of Zoology, University of the Punjab, Quaid-E-Azam Campus
Lahore – Pakistan
E-mail: dr.zafariqbal.pu@gmail.com

INTRODUCTION

Fishes are cold blooded animals and their bodily functions are heavily influenced by water temperature and other external environmental factors like dissolved gasses, pH, osmotic pressure, ions and elements present in water and some may end up in variety of diseases. So fishes like other animals are prone to a variety of diseases. Because disease is an interaction and balance between the host, pathogens and environment, when this balance is disturbed disease may ensue.

The body of the fish is a potential host for many types of pathogens. Though it is difficult to observe diseased fish under water, diseased fish behave quite differently from their fellows. Peculiar signs of its illness are slow and poor response to feeding and external stimuli, swimming in isolation and sometimes on water surface, congregation near falling water, rubbing and scratching against hard objects, and in some cases different postural movements. Diseases related to water quality and toxicity are sometimes hidden leading to an abrupt outbreak and kills a lot of fish if the pond is not properly monitored and maintained. So, fish disease needs a very different approach in understanding behavior and successful management and accordingly its treatment needs to be viewed from different dimensions. Precautionary measures and good management should be at the top but if any fish with abnormal behavior is observed and its treatment is proved difficult, it should be immediately eradicated before this menace spreads in other stock and/ or in adjacent ponds.

TYPES OF DISEASES

Fish diseases are divided into two types as: Infectious and non-infectious diseases.

Infectious diseases are mainly caused by pathogens like viruses, bacteria, fungi or unicellular algae and parasites. The primary pathogens, when present will cause disease when their population proliferates to an uncontrolled level and /or fish is moribund and feeble with loss of its resistance capabilities. Opportunist pathogens are the most common and can be stated as secondary pathogens which become pathogenic under environmental stress.

Non-infectious diseases follow comparatively different routes but sometimes they overlap and these non-infectious factors facilitate the infectious pathogens. However, non-infectious diseases can be fundamentally categorized as; genetic abnormalities, nutritional imbalances, pollutant toxicities, environmental changes and physical injuries or damage. These factors induce stress when they do not fulfill the required criteria for normal fish culture practices, which ultimately activate pathogenic organisms and promote disease, hamper normal activities of fish and cause mortalities if not well controlled or managed in time.

EFFECTS OF FISH DISEASES

The rapid growth of fish farming and intensification of existing fish units has brought and introduced a variety of fish diseases. Diseases present in any living organism disturbs its normal body functioning which prevent fish from eating, retards growth, diminishes reproductive capabilities and deteriorates its flesh quality (Fig.1). Most of the commonly observed fish diseases in carps and other fishes farmed in Pakistan, along with their available and commonly practiced treatments are discussed in the following paragraphs.

BIOSECURITY

Climate change and exchange of organisms are predicted to lead to an increase and spread of new pathogens in humans and wildlife. Many of diseases that are most serious problems in cultured fish are caused by exotic pathogens. On the other hand, worldwide demand for high quality fish products makes disease control an increasingly important issue. Biosecurity refers to the implementation of methods to prevent the transmission of infectious diseases in fish rearing facilities. It also reduces the spread of pathogens that might be endemic to a geographic region. Biosecurity is also important to prevent the escape of pathogens from farm to farm. The general features of an effective biosecurity program apply to all culture systems, whether ponds, cages, raceways or aquaria. Two key methods which are used to maintain biosecurity are pathogen inactivation strategies and inhibiting transmission from one fish to another. However, the relative importance of each component of a biosecurity procedure will vary with fish farming facility, but the general principles will be applied in all cases. The following points must be observed during fish rearing.

- Never introduce fish from other farms.
- Restrict movement of fish between farm sites.
- Observe strict sanitary measures
- Physically separate all units and the diseased fish must be kept in isolated rearing units.
- Feed hygienic food to fish

Pathogens may spread either by the fish or as fomites. The pathogens from fish can be eliminated with antiseptics, while fomites (any agent, such as surfaces or clothing that is capable of absorbing and transmitting the infecting organism of a disease) are inactivated with disinfectants. Fish to fish transmission of pathogens can be minimized by:

- Having separate water supply for each rearing unit.
- Limiting unauthorized access of people to farm
- Keeping separate age classes of fish
- Using disease resistant fish strains
- Stocking pathogen-free stocks in ponds
- Observing quarantine procedures for a new fish population prior to stocking

Viral Diseases of Carps

Viral diseases are of considerable importance in the economic viability of fish production unit and inflict heavy losses if they occur. The common viral diseases are: spring viraemia of carp (SVC), grass carp hemorrhagic septicemia (HSC) and fish pox (carp pox).

1. Spring Viraemia of Carp (SVC)

It is an acute systemic viral disease of common carp reared in intensive culture. The causative agent is *Rhabdovirus carpio*. The disease appears in spring during the first and 2nd year of fish life. The disease is confined to Europe, losses are very high if it outbreaks. The disease also affects other cyprinids including goldfish, grass carp, silver carp as well as some non-cyprinid fishes. The imported goldfish and koi from places with its known presence have shown their susceptibility to this disease. The clinical signs are; oedema, lethargy, enteritis, exophthalmia, and thickening of the swim bladder, petechial hemorrhages in the internal organs, skin and muscle. Mortalities are low at the start but grow steadily depending upon environmental conditions, i.e. low water temperature and level of secondary infection. Prevention is the only remedial measure and is effective where there is no introduction of infected fish in a culture unit.

2. Grass carp haemorrhagic septicaemia (HSC)

Reoviruses have been isolated from some cyprinid species; common carp, black carp, grass carp, chub, tench and golden shiners. Studies in China have demonstrated that the hemorrhagic conditions in carps are associated with a reovirus when it coexists with a second virus. The second virus is tentatively classified as picornavirus. The disease occurs when both the viruses are present. This disease has been reported in the USA in cyprinids (golden shiners) and is of great economic importance in China affecting the grass carp. There are no obvious clinical signs and 1 to 2.5 years old fish are the most susceptible and mortality may reach up to 80%.

3. Fish pox

Fish pox is a common papillomatous condition which occurs on the skin of many species of fish including common carp, goldfish and other cyprinids. Mortality in infected fish is rare. But the skin lesions make the fish unacceptable to the consumers; hence this condition affects the fish trade. Aetiology of fish pox has been associated to some protozoan parasites. However, recently the disease has been suspected to be caused by a herpes type virus, Clinical signs include smooth, white and opaque raised areas on skin. The lesions are 1-2mm diameter and may cover the whole body and fins in heavy infection. Sometimes the lesions progress from simple hyperplasia to papillomatous condition. This is a seasonal infection in most countries, which occurs in winter (below 14º C) in cyprinid and reduces in summer. Non introduction of an infective fish is an effective control. Raising temperature can help in reducing the intensity of lesions. There is no known suitable treatment for this disease.

BACTERIAL DISEASES OF CARPS

Bacterial diseases are common in carps and may equally affect both wild and farmed species. Stress and overcrowding conditions favour their transmission. Some bacterial diseases are due to obligate bacterial pathogens whereas, many pathogenic bacteria are found in commensal association in hosts or freely found in the environment. Both these forms of bacteria become pathogenic when the fish is immunocompromised due to stress and become prone to various infections. Poor water quality, high organic load, contaminated feed and unhygienic conditions are some pre-disposing factors for an outbreak of bacterial diseases in aquatic animals. Bacterial infection can also occur in combination with a primary pathogen or become virulent after the primary pathogen has weakened the fish. The bacteria that cause pathogenecity of carps are mostly "Gram-negative" types.

1. *Pseudomonas* septicemia (Erythroderma)

Bacteria of the *Pseudomonas* spp. are gram negative, non-acid-fast, non-sporing rods with a single polar flagellum, measuring about 2×6.4 µm. *Pseudomonas fluorescens*is a dominant component of freshwater ecosystems and soils and has been associated with septicemia and ulcerative condition also known as erythroderma in a wide range of fishes. It has been considered as a fish spoilage organism as well as primary pathogens, found on the body of fishes. It can infect many fish species, including major carps, common carp and Japanese flounder. *Pseudomonas* infection in fish leads to development of so called Red skin disease, which is prevalent throughout the year particularly when fish is injured during handling and transportation. The clinical signs of diseased fishes include; erosions at the bases of fins, skin lesions and hemorrhages on the surface of the body, loosening of scales, mucous secretion, and superficial to deep ulcers. The occurrence of four species of bacteria; *Pseudomonas anguilliseptica*, *P. fluorescens, P. aeruginosa,* and *Pseudomonas* sp. in *Labeo rohita* and *C. carpio* are reported. *Pseudomonas fluorescens* and *Pseudomonas anguilliseptica* are highly pathogenic to these two species, *L. rohita* and *C. carpio.* These bacteria are present externally on lesions and hemorrhages and internally in and on liver, kidney, spleen and gills of diseased fishes. The bacteria are considered weak pathogens, however severely affected fish die. The infection is self-resolving without antibiotic therapy when the source of stress is removed.

2. Motile *Aeromonas* septicaemia (Fig. 2, 3, 4, 5, 6, 7, 8, 9)

Aeromonas hydrophila is the most common pathogenic species. *Aeromonas* sp. is gram negative, acid fast, non sporing rod with a single polar flagellum, measuring about 1.0-1.5×6.5 µm. *Aeromonas hydrophila*is ubiquitous and is found equally in both freshwater and brackish waters. The bacteria generally cause a systemic hemorrhagic disease in commercial fishes like *L. rohita, Catla catla, Cirrhinus mrigala,* catfish, *Heteropneusstes fossilis* and *Clarias batrachus* and perch *Anabas testudineus.* Earlier *Aeromons hydrophila*, *A. punctata* and *Pseudomonas fluorescens* were considered a cause of “infectious dropsy in carps”. This disease now has been demonstrated to have viral aetiology but the isolation of *A. hydrophila*, *A. punctata* and *P. fluorescens* from diseased carp as secondary pathogens has been reported where they led to clinical disease and mortalities. Clinically fish show lethargy, cutaneous lesions and low mortality. Inflammation and erosion in and around the mouth, with hemorrhaging and necrosis of the fin is common. Exophthalmia and abdominal distension with pinkish-red ascetic fluid liberating from belly when fish is incised. Bacterial septicemia in carps is frequently associated with some common predisposing factors such as; poor environmental conditions, overcrowding, pollution, low oxygen, handling stress, high temperature (above12° C) warm weather. Treatments can be administrated either for prophylactic control or chemotherapeutic measures. Antibiotic therapy can be effective.

3. Bacterial kidney disease (BKD)

Bacterial kidney disease is caused by small, non-motile, gram positive, *Coccobacillus* bacteria *Renibacterium salmoninarum.* It is a serious chronic infection of cultured fishes and is considered as a serious problem in Salmonid farming worldwide and has also been reported in commercial carps. BKD has been reported in different species of freshwater fishes in India. *Labeo rohita* and *Cirrhinus mrigala* were found infected with disease. The infected fishes show clinical signs such as; lethargy; abnormal swimming, exophthalmia, darkening of skin, open lesions and hemorrhages on the body surface. Lesions become ulcers, kidneys become swollen and ultimately are damaged severely. Infected fishes show the occurrence of *R. salmoninarum* in kidney which is the target organ. The bacteria can be identified with a specific fluorescent antibody test, enzymes linked immunosorbent assays (ELISA) or by polymerase chain reaction (PCR).

4. Columnaris disease

Columnaris disease, caused by the bacterium *Flexibacter columnaris* may result in acute or chronic infections in both cold-water and warm-water fishes. It occurs both as external or systemic infections that result in significant losses of hatchery-reared fish, particularly at warm summer temperatures. Epizootics of columnaris disease frequently occur in natural populations and high losses of fish may be observed. Lesions may be observed on the head, fins or gills. The gill tissues are frequently affected and in those cases the condition is termed "bacterial gill disease". When fin damage occurs, it is often the caudal fin that is affected. Two strains of high and low virulence are reported; highly virulent forms attack gill tissue and the latter strains are primarily responsible for cutaneous infections. Bath in dilute copper sulphate solution may be effective.

Miscellaneous bacterial infection in carps

In addition to *Aeromonas* sp. and *Pseudomonas* sp. some gram-positive bacterial genera like *Bacillus* and *Streptococcus* have been reported from carps like goldfish, koi, silver carp and rohu. The infection resulted in damage and erosion of scales and skin, gill damage, reddish boils on intestine, lesion on skin, vent, mouth and operculum. Four gram-positive (*Bacillus, Staphylococcus, Enterococcus, Carnobacterium)* and four gram-negative bacterial genera (*Aeromonas, Acinetobacter, Moraxella, Lactobacillus)* have also been isolated from an ornamental fantail fish.

Fungal Diseases of carps

These diseases occur during winter and primarily are stress related. During harvesting, fish most often get injured; scales dislodged, abrasions, mucus removed, which then becomes an ideal place for fungal attack. Once it anchors, it promptly spreads because of poor resistance in fish during this period. Nevertheless it disappears when temperature rises and fish return to feeding. Fungi have wide range of infections and vary with culture environment and level of management. Many of the fungal genera have a virulence factor which causes fish diseases under favorable predisposing environment. *Saprolegnia* sp. is the most common fungus species and attacks virtually every freshwater fish at least once in its lifetime. Fungal diseases are the third most serious cause of fish loss in aquaculture, after viruses and bacteria. Fungi are known to attack all the life stages of fish from egg to adult fish. Stressed and poorly fed fishes are more susceptible to fungal infection. Fungus has been reported to cause serious diseases in estuarine and freshwater fishes in Australia, Japan and throughout South Asia.

1. Saprolegniasis (*Saprolegnia* sp, *Achyla* sp) (Fig. 10, 11)

Injuries by high stocking density, poor water quality, cold water, and excessive organic matter promote *Saprolegnia* infection in fish. Some species of genus *Saprolegnia* cause infection in salmonids, carps, tilapias, and catfish. *Saprolegnia* (Fig.11) and *Achyla* infection typically reveal cotton wool like fluffy appearance on body and fins of fish. The skin lesions are very common clinical signs. Infection may be superficial or fungus may penetrate deep into musculature. Infection on eyes and gills may lead to rapid spread and even mortality of the stock. Eye infection may result in blindness (Fig. 10) and the fungal hyphae may further penetrate into brain and eventually fish die. Gill infection may interfere with respiration and constrained osmoregulation. However, skin and fin infection are considered less threatening than present on eyes and gills. Mortality of *L. rohita* due to fungal infection has been reported. High mortality in *H. molitrix* has been associated with *Saprolegnia parasitica* and spores and grown up hyphae of *Saprolegnia* sp. have been isolated from farm raised fingerlings of *L. rohita, C. catla* and *Cirrhinus mrigala*. The occurrence of *Saprolegnia* sp. and *Achyla* sp. has also been reported in grass carp, *Ctenopharyngodon idella* and *C. catla. Saprolegnia* infection in eight culturable and 7 non-culturable fishes have been reported in Bangladesh, where *Cirrhinus mrigala* was the most affected species. The chances of occurrence of injuries to fish must be minimized to control infection. Treatment of fish prior to stocking is recommended. *Achyla* has been successfully treated with gentian violet (1.5 ppm) and hydrogen peroxide.

2. Aspergillomycosis (Fig. 12, 13)

This disease is reported in tilapia, *Oreochromis* sp. The causative agents of this disease are *Aspergillus flavus*, *Aspergillus terreus* and *Aspergillus japonicas*. These fungal species infect fish through contaminated feed and in acute conditions may lead to mass mortalities. Poor pond management, injuries to fish, or decomposing organic matter in pond are some other factors which further facilitate fungal infection. Fungal infections have been widely reported in commercial fishes. *Aspergillus*, *Rhizopus*, *Mucor*, *Penicillium*, *Alternaria* and *Blastomyces* are commonly found in six ornamental fishes, (*C. auratus,* its two verities black moor and shubunkin; koi carp, *C. carpio*, platy, *Xiphophorus maculates,* and guppy, *Poecilia reticulate*).

3. Branchiomycosis (Gill Rot)

Branchiomycosis is a serious fungus-like disease of fish and is caused by the genus *Branchimyces.* This disease has caused acute and high mortality in many freshwater fish species of the families, *Cyprindae, Salmonidae, Angullidae, Ictaluridae, Siluridae, Channidae, Cichlidae* and many others. *Branchimyces sanguinis* affects common carp, tench and three-spined stickleback in Europe and *B.demigrans* infects largemouth bass, northern pike, tench and striped bass in Europe and Taiwan. Other fishes reported to be infected with branchiomycosis include, crucian carp, rainbow trout, striped mullet, grey mullet, Japanese eel, snakehead, tilapia and in fingerling and fry of channel catfish. The major carps like *Labeo rohita* and *Catla catla* have also been reported to have branchiomycosis. Clinically the gills may become mottled due to area of thrombosis and ischemia, which results in formation of alternate dark and light regions in the tissue. Both species of *Branchiomyces* cause similar pathology of gills, however *B. demigrans* affects the entire gill as the hyphae penetrate through blood vessels into lumen and *B. sanguinis* infect gill blood vessels. Reducing organic load and lowering water temperature below 20º C in fish holding facility may minimize the attack as no appropriate and suitable treatment is known.

4. Epizootic Ulcerative syndrome (EUS)

EUS has been reported from many freshwater and estuarine fishes worldwide. In Australo-Pacific and in Southern parts of Asia it is a serious and important disease in culturable fishes. The characteristic features of EUS infection which makes it an atypical water mold infection are; 1) the deep ulcers that often penetrate into body cavity and, 2) serious chronic inflammation directed at the water mold component. Some lesions are small and others may be large deep, necrotic up to 25mm in diameter. The mortality can be high and epidemics may develop very quickly. *Aphanomyces invadans* has been reported to cause the typical EUS lesions. However, aeromonads and vibrios are usually present on ulcers which most likely act as secondary invaders. EUS has been reported from Pakistan, India and Bangladesh from culturable carpsin ponds especially fed with river water. The definitive diagnosis of EUS is based on observation of typical histopathology or culture of *A.invadans* from lesions. There is no known treatment of EUS. The improvement of water quality in ponds is suggested.

Parasitic Diseases of Carps

Parasites are those organisms which live at the expense of the host. The presence of these organisms does not always damage the host. The hosts can be *definitive* or *primary* or *final* host, in or on which the parasite reaches to adult stage. An intermediate host is what is also known as secondary or alternate host, on which a parasite spends its larval or immature stage. Parasites may have direct life cycle, (where no intermediate host is present) or an indirect life cycle (where one or more than one intermediate hosts are present).

A wide range of animal groups parasitize fishes during their life cycle. Fish parasites can be Protozoan, Platyhelminths (Monogenean, Digenean, Cestoda); Nematodes; Acanthocephalla; Crustacea; Molluscs; Annelids.

Protozoan Parasites of carp

1. *Ichthyobodo necatrix* (ichthyobodiasis)

This is a flagellate protozoan formally called *Costia*. It is oval in shape (10-20 μm) and is an obligate freshwater parasite. It has one pointed end bearing two flagella, and attacks skin and gills and lacks host specificity. It parasitizes all species of freshwater fish, causing the disease ichthyobodiasis. Fry and fingerlings are more susceptible. However, older cyprinids under poor water quality conditions can be heavily infected. The infected fish become listless and anorexic. In heavy infection, excess mucus production forms a blue-grey film on the body surface and gills. Hyperplasia of epidermal epithelium is common. Lamellar fusion and hyperplasia can occur if the gills are infected. Its pathogenicity is horizontal and is a known pathogen for salmonids. In hatchery this can be treated and removed by application of formalin.

2. *Piscinoodinium pillulare* (Velvet disease)

Piscinoodiniumpillulare is an ectoparasite on skin, gills and fins of cyprinids. It is potentially a serious pathogen of commercial fishes in aquaria, hatchery and rearing facilities. The infection of *P. pillulare* in fish causes "Velvet disease". The life cycle includes parasitic and free - living stages. The parasitic trophont stage may be between 30-100 μm in length. Thousands of trophonts may cause the fish skin to appear with yellow-green spots. Infection results in localized inflammation, hemorrhages on skin and in heavy infection sloughing of the tissue may occur. In case of gill infections of fish by *P. pillulare,* induces hyperplasia and obstruction of respiratory surface and heavily infected fish may succumb. Sodium chloride and copper sulphate baths are suitable for controlling and eradicating this parasite.

3. ***Ichthyophthirius multifiliis*** **(Ichthyophthiriasis, white spot disease) (Fig.14)**

Ichthyophthirius multifiliis (Fig. 14) is a ciliate protozoan parasite and it causes white spot disease (Ich). It is a potential threat to cyprinids. This is a non-host specific parasite, which resides under the skin on fins and gills. The life cycle of *I.multifiliis*is direct and involves both parasitic and free living stages. A parasite may reach up to 1mm in diameter (trophant stage) that has a characteristic crescent shaped mega-nucleus. Infection on skin and fins results in irritation, detachment of epidermis and mucus. Infection on gills causes gill damage and respiratory stress. In heavy outbreaks 100% mortality can occur. It is difficult to treat this disease because the parasite does not live on the surface of the body but under the epithelium and therefore cannot be reached by conventional treatment. The pond should be dried and disinfected and entry of wild fish into rearing pond should be restricted.

4. ***Trichodina pediculatus*****. (Trichodinosis) (Fig. 15)**

Trichodina (Fig. 15) is a saucer shaped ciliate (40-60 μm in diameter). It is an ectoparasite. *Trichodinapediculatus* has been reported from *H.molitrix* and *C.mrigala.* It moves on skin, fins and gills of fish with the help of tooth like structures called denticles. It feeds on detritus and other debris present on the surface of the fish. It is neither host nor site specific. Trichodinosis is a mild disease but typically shows chronic morbidity, but can cause significant loss in young fishes. Clinical signs include white patches on skin surface and excess mucus exudates on the gills. Infection of this parasite on fish causes irritation on skin, resulting in hyperplasia of the epithelium. Heavy infection on gills results in severe gill damage and respiratory stress and even proves life threatening with low level mortalities. The incidence of mortalities from disease is higher in young fishes. The parasite may be removed from the hatcheries and rearing facilities by an external chemical treatments like formalin, Acetic acid and salt bath.

5 *Chilodonella piscicola* (Chilodonellosis)

Chilodonella piscicola is a pear shaped ciliated protozoan nearly 50 µm in length. The parasite reproduces by binary fission. It can survive below 5º C. It can cause high morbidity and mortality in freshwater fishes by attacking skin and gills of the fish. It has been reported from goldfish, common carp, and major carps. This parasite infects virtually all freshwater fishes especially fingerlings. The parasite causes host reactions such as mucus secretion, epithelial cell necrosis and ulceration. Infected skin is vulnerable to secondary infection under influence of water pollution. Heavy gill infection results in severe gill damage and respiratory stress, however, it can be treated by an external chemical treatments like formalin, Acetic acid and salt bath.

6. *Epistylis* sp.

Epistylis sp. is the most common colonial ciliate. It is very common in South Asian countries in aquarium and pond fishes and is associated with high levels of organic material in ponds. Large numbers of these ciliates attach on skin, gills arches and in mouth of carps in ponds. They attach to the fish host or other substrate by a 1mm long, non-contractile stalk. The parasite does not feed on fish but use the fish as an attachment site. The site of attachment on fish by *Epistylis* develops lesions on the attachment site that become inflamed and necrotic and eventually ulcerated under heavy infection. All species of salmonids are susceptible and eggs of catfish and major carps may also be affected. *Epistylis* infection in cultured catfishes in Brazil is an emerging disease. Control of *Epistylis* infection is by formalin bath and salt bath.

7. *Apiosoma* spp. (syn. *Glostella* spp.)

Apiosoma is goblet shaped 30x50 µm which has flat attachment base and cilia at the anterior end. They attack skin and gills of fish. They are solitary rather than colony forming. Heavy infection causes inflammation, necrosis and ulceration of skin and damage of gill tissue. *Apisomasie wingi* and *A. filiformis* are found on cyprinids and recorded from minnows. Control is by formalin bath and salt bath.

Monogenean parasites of fishes

The most common parasites of freshwater fishes are two types of monogeneans which are typical trematode parasites of gills and skin and are generally host specific. Dactylogyrids are gill parasites while gyrodactylids live on skin and fins.

8. *Dactylogyrus extensus,*

Dactylogyrus sp. (Fig. 16, 16A) is oviparous and has high reproductive rate. It has a short direct life cycle which facilitates it to spread rapidly in fish rearing facilities. The transmission of monogeneans has been reported to increase under poor pond management conditions. *Dactylogyrus extensus* is known as gill fluke and is a common parasite on gills of fishes. It feeds on dermal and gill debris. *Dactylogyrus* infections to gill epithelium invite secondary bacterial, fungal, protozoan infection on gill surface. *Dactylogyrus vastator* causes hyperplasia of gill epithelium and deformation of gill lamellae. *Dactylogyrus extensus* occur between the secondary lamellae and cause damage at the site of attachment. Heavy infection results in trauma and injuries on gill surface, lamellar hyperplasia, and excessive mucus production. The ultimate effects are impaired respiration and stressed fish. Dactylogyrid scan be controlled by using Paraziquentel and Trichlorfon. Draining and disinfection of pond is also suggested.

9. ***Gyrodactylus* sp.**

This parasite is (Fig. 17, 17A) small and transparent, and less than 1mm in size. It is viviparous and its short direct life cycle facilitates it to spread rapidly in fish rearing facilities. *Gyrodactylus* sp. or skin fluke is less pathogenic compared to *Dactylogyrus* sp. Some species of *Gyrodactylus; G. katharinire, G. crysoleucas, G. elegens, G. ctenopharyngodonis* infect cyprinids. Young fishes are more susceptible. Damage to skin, scales and epithelium at the point of attachment of the parasite is prominent. These form foci for entrance of secondary infection, though may be mild. The fish infected with *Gyrodactylus* sp. may develop white to grey whitish areas of thick mucus on skin. Infection also emaciates the fish which may ultimately die. Formalin and / or potassium permanganate baths may be effective.

Digenean Parasites of carp

10. ***Posthodiplostomum cuticola*** **(Black spot disease, Posthodiplostomasis) (Fig. 18)**

Posthodiplostomum cuticola is a digenetic fluke, the adult of which lives in the intestine of piscivorous birdssuch as; common heron, *Ardeacinerea*. The eggs of *P. cuticola* are released into water with bird's feces and hatch into free living larvae, miracidia. Development takes place in aquatic snail *Planorbis* sp. frommiracidiato furcocercaria. Furcocercaria burrows into the skin and fins of the fish, the second intermediate host. The infection in fish by metacercaria, results in the formation of black spots on the skin and fins of fry. Juvenile and young fish are more susceptible to infection. Once the infected fish is eaten by bird, the metacercariae develops into an adult fluke in the intestine of the bird. Black spot disease is common in cyprinids and is also reported in *L. rohita*. Ponds with earthen bottom are very suitable for occurrence of black spot disease. Infected fish shows clinical signs; weight reduction, backbone and musculature deformation, kidney and liver dystrophy, fish does not eat and it often dies. Habitat degradation increases the incidence of black spot disease. Heavy prevalence of *P. cuticola* infection in fish is associated with thick vegetation in water bodies. The control of snails and fish eating birds is essential to minimize black spot disease. Further, infection can be controlled but complete eradication takes years.

11. *Sanguinicola inermis* (sanguinicoliasis)

Sanguinicola inermis lives in the circulatory system of carps. It is slender shaped and more than 1 mm long. *Sangunicola* cause severe disease in fry and two year old carp. The disease is caused by obstruction of the capillaries of the gills and kidneys with eggs of the parasites. The branchial form of the disease occurs mainly in fry and yearlings and renal forms appear mainly in older fish. The obstruction of the capillaries of the gills may cause destruction of filament and the damaged part of gill disappears. The eggs of the parasites block the vessels of the kidney, which affect their function and cause edema in the body cavity and blistering scales. Disease can be controlled by eradication of snails by drying pond or by chemical treatment.

Crustacean parasites of carps

12. *Lernaea cyprinacea* (lernaeasis) (Fig. 19)

*Lernaea cyprincea*isa copepod parasite and is common on freshwater fishes. It is distributed in Asia, Africa, Asia, North America and Europe and recently been reported in native fishes from Brazil and Western Australia. *Lernaea cyprinacea* is more prevalent in still and slow flowing water than in fast flowing streams. Adult female of *L.cyprinacea,* causes lernaeasis. It lacks host specificity to such an extent that it can infect all freshwater fish. The red sores, ulceration and hemorrhages caused by *L. cyprinacea* on fish skin are prone to secondary bacterial and fungal infection. Young fish are most at risk of death. *Lernaea* can penetrate the visceral cavity, including heart and resulting peritonitis and death. Among the culturable carps young *Catla catla* is found to be more susceptible to infection Heavy prevalence of *Lernaea* sp. has been reported in tilapia of Lake Victoria which has been associated to growing environmental stress. Mortality due to lernaeasis in farmed fish has been reported. *Lernaea cyprinacea* has been reported in major and Chinese carps in Pakistan. The five carp species, *L. rohita, C.catla*, *Cirrhinus mrigala, Ctenopharyngodon idella*, *Hypophthalmichthys molitrix* and *Aristichthys nobilis* are the common hosts of *L.cyprinacea* and other *Lernaea* spp. *Catla catla*, *L. rohita* and *C. idella,* showed high prevalence and mean intensity compared to *H. molitrix*. In addition to *L. cyprinacea*; *Lernaea polymorpha*; *Lernaea oryzophila* were found on *L. rohita* and *C. idella. Lernaea ctenopharyngodonis* was found on *C. idella* only. The abdomen and the base of fins are the most common site of attachment of *Lernaea.* Organophosphate with common name "Thunder", Tender, Baytex and Dipterex are commonly used to treat lernaeasis in these fish species.

12. *Argulus foliaceus* (argulosis) (Fig. 20, 21)

*Argulus foliaceus*is a crustacean parasite of cyprinids. *Argulus foliaceus* infection produces ulceration on the skin of the fish and facilitates other pathogens to attack the host. Low infection may lead to epizootics in densely held fish populations, which causes large mortalities. Fish kill due to argulosis has been reported from fish hatcheries and nurseries, rearing ponds, fishing lakes, in home and public aquariums. Argulosis is a threat to commercial fisheries; both in warm and cold waters. *Argulus* spp. represents potentially a serious pathogen. High infection up to 800-1000 parasites / fish may cause mortality in *C. carpio* and other carps. *Argulus* infects tilapia, *Oreochromis niloticus*, rainbow trout, *Oncorhynchus mykiss* and *C. carpio* and goldfish, rudd, *Scardinius erythrophthalmus*. *Argulus inducus, A. japonicas* and *Argulus siamenses* sometimes cause mortality in major and Chinese carps. *Argulus foliaceus* and *Argulus bengalensis* infect 11 freshwater fish species in Bangladesh. The low host specificity of fish lice make it more dangerous and it may infect any freshwater fish.

13. *Ergasilus sieboldi* (ergasilasis)

Ergasilasis is caused by the female of genus *Ergasilus*. There are two species *E. sieboldi* and *E. briani* which attach to the outer side of gills and between the filaments respectively. The mature female has a pear shaped body, 1.0- 1.5 mm long. Few hundred to thousands parasites may be present on individual fish. The parasite feeds on gill tissue and blood. Infection occurs in July-August. The parasite causes deformation of the gill filament, compression of the blood vessels, and necrosis of gill tissues. Secondary infection by fungus and bacteria are common. Host fish suffer osmoregulatory problems. The mortality is often very high. The effective way of eradication of *E. sieboldi* is by draining the pond completely and applying lime and leaving it dry for 6 months from winter to spring.

Some other parasites and their sites of infection are: Myxozoa (*Myxobolus rohitae* from *H.molitrix* and *C.mrigala*) Digenean (*Phyllodidtomum agnotum* from stomach of *C. mrigala*, *L.rohita, C.catla; Eucreadium* sp. from intestine in these fishes), Acanthocephala (*Pallisentis* sp. from intestine of *H. molitrix, C.mrigala*); Nematoda (*Camallanus ophiocephali* from intestine in *C.mrigala*), Annelida (*Hirudo* sp. from skin and fin); cestode (*Ligula intestinalis* in intestine of *C. catla*) and many others.

Annelid parasites of carps

Leeches are occasionally seen in wild and pond fishes and even in ornamental fishes like goldfish. The genus *Piscicola* is a common parasite of fish. Leeches attach on the skin, gills and oral cavity of fish with the help of its oral sucker. Infection results in anemia and small red lesion on the body. Pathology depends upon the amount of the blood taken. Heavy infection may cause serious anemia. The leeches also act as carrier for microbes and blood parasites during feeding. Leeches can be treated with a single dose of organophosphate.

TABLE 1.Common freshwater diseases, diagnostic and treatments for famed fishes.

Symptoms	Diagnosis	Treatment
Grayish-white film on skin, damaged fins, ulcers, yellow to gray patches on gills, tissue on head may be eaten away.	*Columnaris* (Cotton Wool Disease)	Must be treated immediately with Over-the-counter antibiotic medications. Very contagious disinfect tank, rocks, net, etc.
Swelling of head, bulging eyes.	Corneybacteriosis	Over the Counter (OTC) antibiotics such as penicillin and tetracycline.
Swelling of abdomen, raised scales around swollen area.	Dropsy (Malawi Bloat) may be caused by internal bacterial infection (if swelling is sudden), parasites, or cancer (if swelling is gradual).	Add 1/8 teaspoon of Epsom salt for every 5 gallons of water and monitor for two weeks. Check for signs of bacterial infection or parasites for further treatment.
Ragged or decaying fins.	Finrot	Check pH and correct as needed. If level is normal, use OTC antibiotic for fin or tail rot.

Inactivity, loss of color or appetite, weight loss, skin defects.	Fish Tuberculosis	Human strength TB medication may help in early stages. Contagious disinfect tank, rocks, net, etc. to prevent transmission. Wash hands and surfaces well.
Erratic swimming, bloating or swelling in body, black patches on body or fins.	Myxobacteriosis -- rare	Medications, if any, are difficult to come by. Keep up on water maintenance to prevent it.
Sluggishness, lack of appetite, fin damage, reddish discoloration, bulging eyes, clamped fins	Septicemia	Antibiotic treatment in food form is required.
White or gray fungus on eyes.	Cataracts	OTC medication for fungus.
White or gray patches resembling cotton, excess mucus.	Mouth or Body Fungus	OTC medication for fungus. Usually added to water, but may need direct application.
White cotton-like patches on fins, body, or mouth.	True Fungus (*Saprolegnia*)	OTC medication for fungus. Check for symptoms of other illnesses.
Small string-like worms visible on fish, or burrowed in skin.	Anchor Worm	Over-the-counter medication for parasites.
Bluish-white film on body, strained breathing caused by gill damage, peeling skin.	*Chilodonella*	Salt treatment (see below).
Weight loss, strained breathing.	Copepods	Over the-Counter medication for parasites, also fungal treatment for possible secondary

		infection on damaged gills
White film, reddened areas on body, abnormal swimming, scratching, folded fins.	*Costia* (Slime Disease)	Must be treated quickly. Raise water temperature and use OTC medication for parasites. Salt treatment may work, as well.
Weight loss, abnormal swimming, generally looks very ill. Will accompany or follow leech infestation.	Blood Flagellates (Sleeping Sickness) rare	Salt treatment can be used to kill leeches, but may not cure flagellates.
Sluggishness, flashing, spider web lesions on skin, color loss, reddened fins, drooping fins, fin damage.	Skin Flukes (*Gyrodactylus*)	OTC medication for parasites
Lack of appetite, weight loss, small holes or eroding pits appearing in the head.	Hole in Head Disease (Hexamita) more common in cichlids	OTC medication for Hole in Head Disease.
Scratching, white salt-like spots starting on head and spreading over whole body, rapid breathing, cloudiness on eyes or fins.	Ich (*Ichtyophthirius*) very common	OTC medication for Ich or Ick.
Scratching, small worms hanging from body.	Leech	Salt treatment or OTC medication for parasites.
Scratching, green to brown lice (up to inch) visible on skin.	Lice	OTC treatment for parasites.
Erratic swimming, weight loss, loss of color.	Neon Tetra Disease mostly affects tetras, danios, and barbs	Treatment is difficult look for a medication that treats gram-negative bacteria or with nalidixic

		acid as the active ingredient.
Darting, scratching, small yellow to white spots dusting skin.	*Oodinium*	OTC treatment for parasites.
Cloudy appearance on skin, red patches on skin where parasite has bitten.	*Trichodina* -- predominately freshwater	Salt treatment.
Red or bloody gills, gasping for air.	Ammonia Poisoning	No treatment. Regular water testing and maintenance will prevent it.
Small dark spots on fins and body.	Black Spot	OTC medication for parasites. Spots (cysts) may remain after treatment.
Cloudy white appearance to one or both eyes.	Cloudy Eye	Check for symptoms of another illness like velvet, ich, or tuberculosis. Treat with OTC medication.
String of feces hanging from fish, swollen abdomen, sluggishness, disinterest in food, off-balance swimming.	Constipation	Stop feeding for 2-3 days and continue with a more varied diet including live and plant-based foods.
Small white spots that get larger over time possibly with black streaks.	Fish Pox	No treatment. Keep up on water maintenance and symptoms should cease after about 10-12 weeks.

Difficulty swimming, swimming upside-down, floating, unable to surface. Do not confuse with swim bladder disease.	Flip over	Air can be removed from swim bladder by a veterinarian. Surgery is also a possibility in larger fish. Check for signs of internal infection or parasites and treat as necessary.
Reddening on or under skin, sudden abnormal behavior.	Inflammation	OTC antibiotic treatment.
Unusually bulging of one or both eyes.	Pop-eye (Exophthalmia)	OTC medication for bacterial infections and/or parasites. Check for other symptoms of bacterial or parasitic infections.
Fish struggles to swim, may float with head tipped down, or have difficulty surfacing, no balance, etc. May occur after eating.	Swim Bladder Disease	Stop feeding for 3-4 days. If symptoms persist, feed the affected fish a small amount of fresh spinach or a green pea without the skin (laxatives).
Swelling or distention for internal tumors, external can be seen growing on skin.	Tumors	Usually incurable. Consult a veterinarian about potassium iodide treatment for thyroid tumors.
Sluggishness, lack of appetite, open sores with red edges, possible fin rot.	Ulcers	OTC medication for bacterial infections.
Scratching, small gold to white spots, loss of color, weight loss, difficulty breathing due to gill damage.	Velvet (Gold Dust Disease)	OTC medication for parasites.

Fig.1. *Labeo rohita* A. healthy, B. mildly infected, C. diseased)
(Courtesy of Dr. Zafar Iqbal)

Fig.2. *Ctenopharyngodon idella* with early stage of *Aeromonas* septicemia
(courtesy of Dr. Zafar Iqbal)

Fig.3.*Ctenopharyngodon idella* with infection of *Aeromonas* septicemia (courtesy of Dr. Zafar Iqbal).

Fig.4.*Cirrhinus mrigala* with severe *Aeromonas* septicemia (courtesy of Dr. Zafar Iqbal)

Fig. 5. *Labeo rohita* with skin lesions and damaged dorsal fin. (Courtesy of Fish Disease and Health management Lab. P.U, Lahore).

Fig. 6. *Labeo rohita* with skin lesion above anal fin and infected dorsal fin. (courtesy of Fish Disease and Health management Lab. P.U, Lahore).

Fig. 7. *Labeo rohita* with lesions on operculum and base of pectoral fin. (courtesy of Fish Disease and Health management Lab. P.U, Lahore).

Fig. 8. Silver carp with lesion on lower jaw and exophthalmia of eye. (courtesy of Fish Disease and Health management Lab. P.U, Lahore).

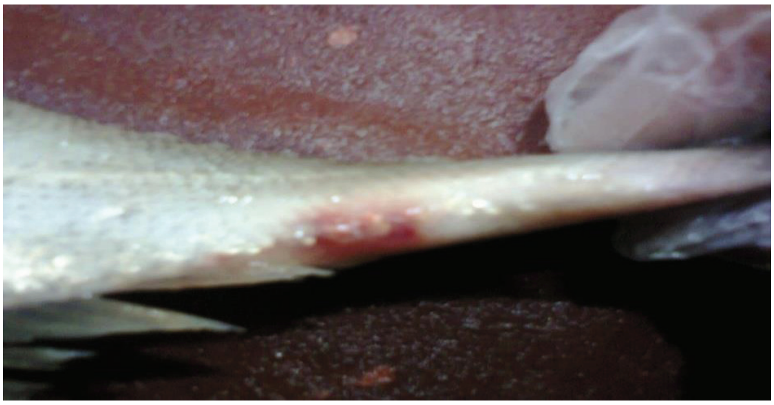
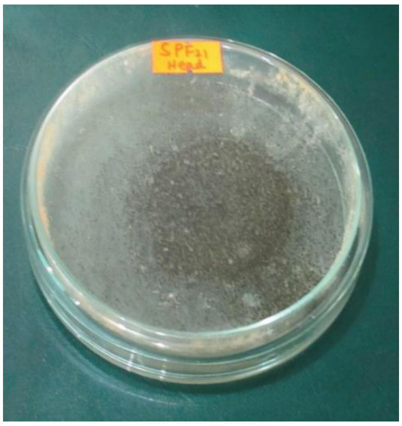


Fig.9. Silver carp with skin lesion near anal fin (courtesy of Fish Disease and Health management Lab. P.U, Lahore).

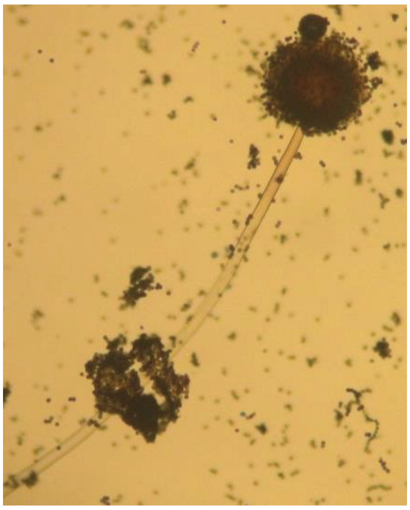
Fig.10. *Labeo rohita* fingerling having severe *Saprolegnia* sp. infection. The eye is totally covered with fungal hyphae and caudal fin is eroded completely (courtesy of Dr. Zafar Iqbal).

Fig.11.Unstained *Saprolegnia* hyphae taken from *Labeo rohita*
(courtesy of Dr. Zafar Iqbal)

Fig.12.*Hypophthalmichthys molitrix* with lesions on the body surface
(courtesy of Fish Disease and Health management Lab. P.U, Lahore).



A **B**



C

Fig.13A. Pure culture of fungal colony from head on SDA. *Aspergillus* spp. (B, C) isolated from colony A (Sporangium with hyphae, 100X) (courtesy of Fish Disease and Health management Lab. P.U, Lahore).

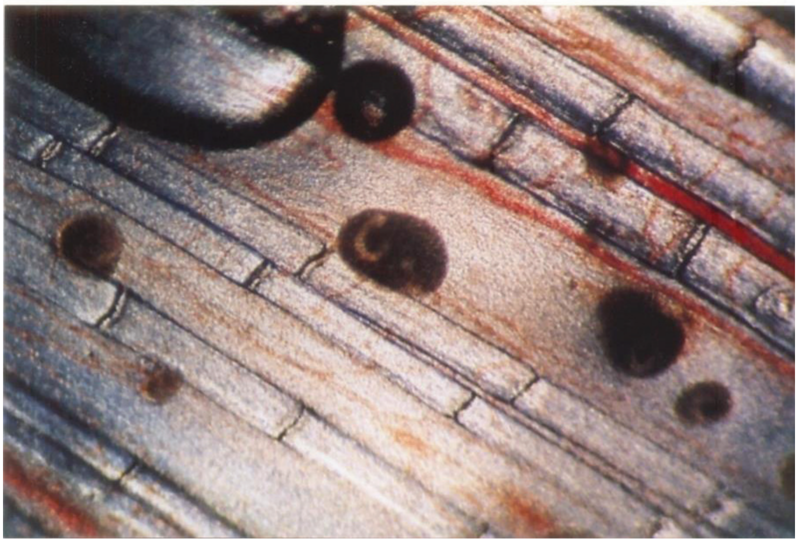


Fig.14.*Ichthyophthirius multifiliis* present on caudal fin of goldfish (courtesy of Fish Disease and Health management Lab. P.U, Lahore).

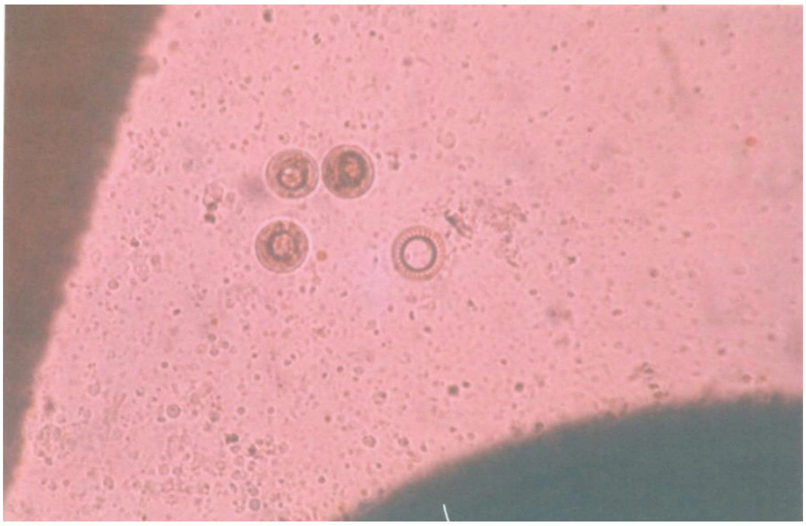


Fig.15. *Trichodina* sp. from skin of goldfish (courtesy of Fish Disease and Health management Lab. P.U, Lahore).

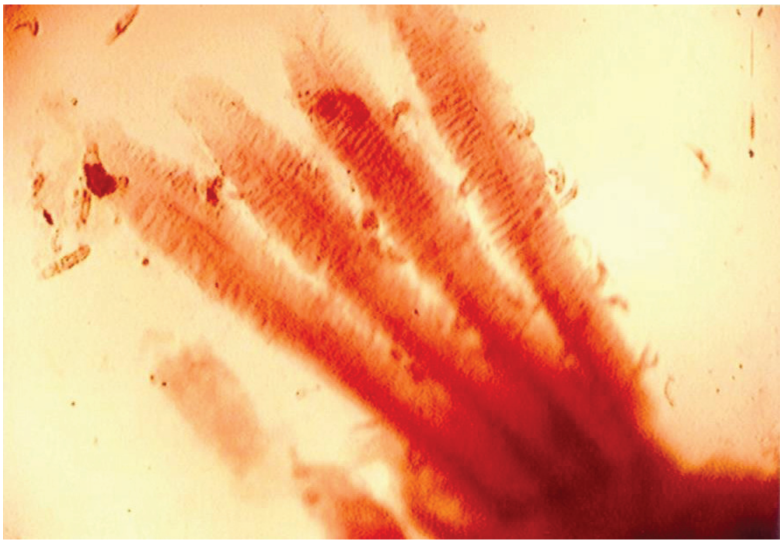


Fig. 16. Heavy infection of *Dactylogyrus* sp. on gill filaments of *Labeo rohita* (courtesy of Fish Disease and Health management Lab. P.U, Lahore).

Fig. 16A. Severe gill infection of fantail, a variety of goldfish by *Dactylogyrus* sp. The gill filaments are totally damaged (courtesy of Fish Disease and Health management Lab. P. U, Lahore).

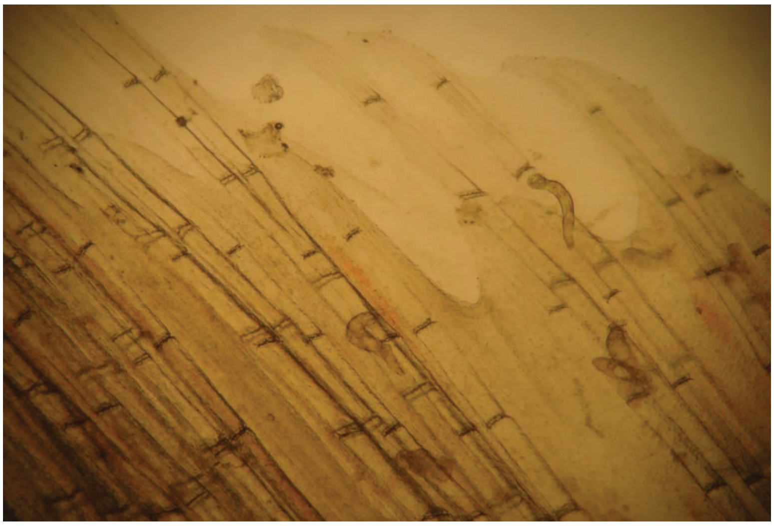


Fig.17.Multiple *Gyrodactylus* sp. infection on caudal fin of goldfish (courtesy of Fish Disease and Health management Lab. P.U, Lahore).

Fig.17A.*Gyrodactylus* sp. attached on pectoral fin of fantail, a variety of goldfish. The marginal and median hooks are very clear (courtesy of Fish Disease and Health Management Lab. P.U, Lahore).

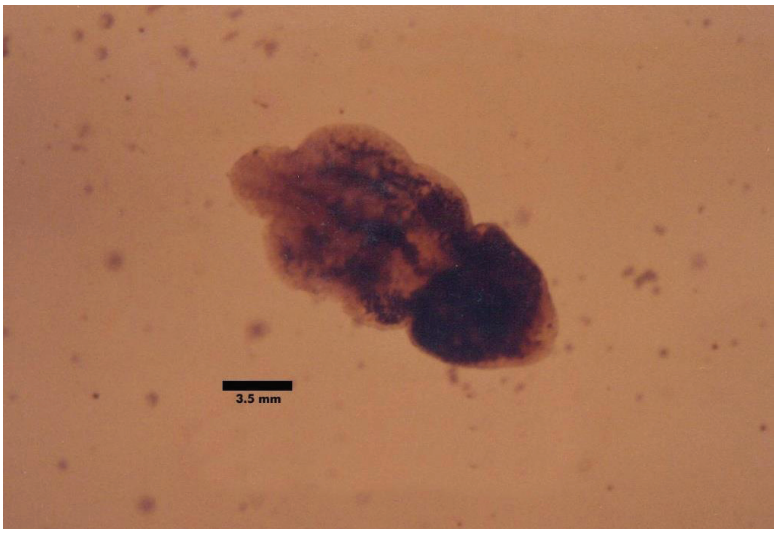


Fig.18.Metacercaria of *Posthodiplostomum cuticola* from *Labeo rohita* fry (courtesy of Dr. Zafar Iqbal).

Fig. 19.*Cirrhinus mrigala* heavily infected by *Lernaea cyprinacea* (courtesy of Dr. Zafar Iqbal).

Fig.20. Black moor, *Carassius auratus* with skin lesion (4.5 x 3.5cm) (courtesy of Fish Disease and Health management Lab. P.U, Lahore).

Fig. 21. *Argulus foliaceus* from koi carp (courtesy of Fish Disease and Health Management Lab. P.U, Lahore).

Acknowledgements

This chapter would have not been completed without the very valuable contribution of my colleagues and students, whose work and photographs have been included here. Some of them are Imtiaz K. Minhas, Sonia K. Shukerova, M. Naeem Khan, Muhammad Asghar and students are Fauzia Haroon, Uzma Sheikh, Uzma Najam, Saira Saleemi, Zil-E-Huma, Farah Ansar, Rabia Mughal, Andella Shafaqat, Ifat Rani, Rubaba. Ramsha Sajjid, Uzma Hussain, Rabia Mumtaz, Beenish G. Rehman, Maryam Abdul Bark. The consultation of reference books and research papers in preparation of this work is gratefully acknowledged.

As many people made significant contribution to this chapter, any errors of omission remain entirely my own responsibility.

Dr. Zafar Iqbal, Ph. D.

References

Viral Disease References

Bucke, D and Finlay, J. 1979. Identification of spring viraemia in *Cyprinus carpio* L. in Great Britain. Veterinary Record; 104: 69-71.

Hadrick, R.P., Groff, J.M., Okihiro, M.S. and McDowell, T.S. 1990.

Herpes viruses detected in papillomatous skin growth of koi carp (*Cyprinus carpio).* J. Wildlife Diseases, 26: 44-57

Bacterial Diseases References

Austin, B and Austin, D.A. (eds), 1987. Bacterial Fish Pathogens: Diseases in farmed and wild fishes. John Wiley and Sons Ltd., Chichester, UK.

Farah, Ansar, 2013. Bacterial infection in imported shubunk in, a variety of goldfish, *Carassius auratus* L. MS. Thesis. Department of Zoology, University of the Punjab, Lahore, Pakistan. pp.93

Minhas, I. K., Iqbal, Z and Khan, M.N., 2001C. Occurrence of abdominal dropsy in culturable fish species in Punjab. Proceedings of the *21st Pakistan Congress of Zoology (International),* held at Agriculture University Faisalabad, 15-17 March 2001; (21): 169-174.

Malathi, K and Rajendran, K., 2012. Isolation of fungi and bacteria from various tissues of freshwater fish *Chana punctatus* (Bloch). *Asian J.Micro.Biotech. Environ. Sciences,* 14(4): 553-556.

Mastan, S.A. Pseudomonas Septicemia in *Labeo rohita (Ham.)* and *Cyprinus carpio* (Linn.) in Andhra Pradesh-Natural occurrence and artificial challenge. Int. J. Pharmacy & Pharmaceutical Sciences, Supplement 2, Vol.5. P.564.

Mastan, S.A and Ahmed, M. Osman, 2013. Bacterial Kidney Diseases (BKD) in Indian Major carp fishes, *Labeo rohita* (Ham.) and *Cirrhinus mrigala* (Ham.) –Natural occurrence and artificial challenge. Asian J. Pharmaceutical & Clinical Research, 6 (3). Abstract.

Zil-E-Huma, 2013. Isolation and Characterization of bacterial pathogens from some freshwater Fishes. M.Phil Thesis. Department of Zoology, University of the Punjab, Lahore, Pakistan. pp.112

Fungal Diseases References

Anderson, B. and Thrane, U., 2006. Food-borne fungi in Fruit and Cereals and their production of mycotoxins. *In*: Hocking A. D., Samson R. A., Pitt J.I and Thrane U. (Eds.) *Advances in Food Mycology*. Springer. USA, pp: 137.

Bangyeekhun, E. and M. A. Sylvie, 2001. Characterization of *Saprolegnia* sp. isolates from Channel Catfish. *Diseases of Aquatic Organisms* 45(1):53-59.

Bruno, D.W and Woo, B. P., 1999. *Saprolegnia* and other Oomycetes. *In: Fish Diseases and Disorders*, Volume 3, Viral, Bacterial and Fungal Infections. (Eds. P.T.K. Woo and D.W. C. T., Cab International UK, pp 599-669.

Chauhan, R., 2012. Study on certain fungal diseases in cultureable and non-culturable species of fishes of Upper lake Bhopal. *J. Chem. Biol. Physical. Sci*. 2(4): 1810-1815.

Das, S.K., K, Mumu., A, Das., I, Shakuntala, R.K Das, S.V. Ngachan, and S. K. Mjhi, 2012. Studies on the identification and control of a pathogen *Saprolegnia* in selected Indian major carp fingerlings at mild altitude. *J. Envior. Biol*. 33, 545-549.

Fayioye, O.O., Fagbohun, T.R and Olubanjo, O.O., 2008. Fungal infestation and nutrient quality of traditionally smoke-dried freshwater fish. *Turk. J. Fish Aqua. Sci*, **8**: 7-13.

Firoozbakhsh, F., Ebranhimzadeh Morusavi, E. H.A and Khosravi, A.R., 2005. Isolation and Identification of Pathogenic and saprophytic fungi from gill lesion in cultivated cyprinids (common carp, silver carp and grass carp). *J. Fac. Vet. Med. Univ. Tehran*, **60**:15-19.

Haroon, F., Z. Iqbal, K. Pervaiz and A. N. Khalid. (2014 **i**n Press**)** Incidence of Fungal Infection of Freshwater Ornamental Fish in Pakistan. *Int. J. Agri. Biol*, Vol.16 (3): xx-xxx

Hatai, K., 1989. Fungal pathogens/parasites of aquatic animals. In: Methods for Microbiological Examination of Fish and shellfish (eds. B &D.A. Austin).pp 240-2772. Ellis Harwood Ltd. Chichester, UK.

Hussein, M.M., K. Hatai and T. Nomura., 2001. Saprolegniasis in salmonids and their eggs in Japan. *J. Wildl. Dis*., 37: 204-207.

Iqbal, Z, Asghar, M and Rubaba. 2012a. Saprolegniasis in two commercially important carps. *Pak. J. Zool*., (44)2: 515-520.

Iqbal, Z., U. Sheikh and R. Mughal, 2012b. Fungal infection in some economically important freshwater Fishes. *Pakistan Vet. J*, 32: 422–426.

Iqbal, Z. and R. Sajjid, 2013a. Some Pathogenic Fungi Parasitizing two exotic tropical Ornamental Fishes. *Int. J. Agri. Biol,* 15: 595–598.

Iqbal, Z. and R. Mumtaz, 2013b. Some fungal pathogens of an ornamental fish, Black Moor (*Carassius auratus* L.). *Eur. J. Vet. Med,*2(1): 1-10

Jalilpoor, J., Mosouleh, S.A and Masoumzadeh, M., 2006. Fungal flora in *Acipenser persicus* eggs with particular emphasis on *Saprolegnia* sp. (Oomycetes) and mortality during mass incubation at the Shahid Behesti hatchery. *J. App. Icthyol,* 22(suppl.1): 265-268.

Jubeen, F., I. A.Bhatti., U, Maqbool and S.Mahboob, 2012. Fungal incidence, aflotoxin B1, tocopherols and fatty acids dynamics in ground and tree nuts during storage at two moisture levels. *Int. J. Agri. Biol*., 14:521-527.

Krishna, L., Gupta, V.K., Katoch, R.C and Singh, D., 1990. Saprolegniasis in Indian major carps- An investigation. *Indian Vet. J.,* 67: 554-555.

Loan, L.T.T., Phung, V.H., Thanh, T.P and Huyen., N.T., 2006. Experimental screening of some pharmaceutical chemical compound affecting *Achyla* sp. isolated from catfish eggs. Proc.Int. W/shop Biotech. Agri. held at Nong Lam University of HoChi Minh City, Oct.20- 21, 2006.

Neish, G.A., 1997. Observations on Saprolegniasis of adult sockeye salmon, *Oncorhynchus nerka* (Walbaum).*J.Fish.Biol.,*10: 513-522.

Olufemi, B.E., Anguis, C and Roberts, R.J., 1983. The Aspergillomycosis in intensively cultured tilapia from Kenya. *Vet. Rec*., 112: 203-204.

Pailwal, P. and S.C. Sati, 2009. Distribution of Aquatic fungi in relation to physiochemical factors of Kosi River in Kumaun Himalaya. *Nat. Sci*., 7(3): 70-74.

Refai, M., K. Laila., A. Mohamed., M. Kenawy and El. SMS. Shimaa. 2010. The assessment of Mycotic settlement of freshwater fishes in Egypt.*J. Amer. Sci.,* 6(11): 595-602

Siddique, M.M.R., Basher, M.A., M.A. Hussain and A.S.M. Kibria, 2009. Fungal Disease of Freshwater Fishes in Natore District of Bangladesh. *J. Bangla. Agri. Uni*., 7(1): 157-162.

Shahbazain, N., Ebrahimzadeh, M., Soltani, M., Khosravi, A.R., Mirzagai, Sand I. Sharifpour, I., 2010. Fungal contamination in rainbow trout eggs in Kermanshah Province propagation with emphasis on Saprolegniaceae. *Iranian J. Fish Sci*, 9: 151-160.

Srivastava, R.C., 2009. Fish Mycopathology. Today and Tomorrow's Printers and Publishers New Dehli, pp: 103.

Tavares-Dias, M., Lemos. G. R. J. & Martin. L.M., 2010. Parasitic fauna of eight species of ornamental freshwater fish species from the middle Negro River in the Brazilian Amazon Region. *Rev. Bras. Parasitol. Vet., Jabotical.,* 19(2) : 29-33.

Parasitic Reference

Alam, M.M., Khan, M.A., Husain, MA.,Moumita, D., Maziaz, AG and Simon, K.D., 2012. Intensity of parasitic infestation in silver carp, *Hypophthalmicthys molitrix*. *J. Zhejiang Uni. Sci. B.* 13(2): 1024-28

Bond, N. R., 2004. Observations on the effects of the Introduced parasite *Lernaea cyprinacea* on a lowland population of a small native fish, mountain *Galaxias olidus*. *Victor. Naturalist,* 121:194-198.

Chanda, M., Paul, M., Maity, J., Dash, G., Gupta, D., Gupta, S. S. & Patra, B. C., 2011. Ornamental fish goldfish, *Carassius auratus* related parasites in three district of West Bengal, India. *Chron Young Sci*. 2:51-4.

Clayton, G. M. & Price, D. J., 1992. Interspecific and intraspecific variation in resistance to Ichthyophthriasis among poeciliid and goodeid fishes. *J. Fish Biol.,* 40: 445-453.

Delwer, M.D., Hassan, A.N.G.M., Haque, M.E., Ali, M.S., Ali, M.H and Barman, A.C., 2010. Parasitic diseases of Indian major cars in Rajshahi district of Bangladesh. J. Agrofor. Environment, 3(2): 167-170.

Durborow, M. R., Mitchell, J. A. & Cosby, M. D., 1998. Ich (White Spot Disease) SRAC Publication No. 476. USA

El-Dien, A. H. & Abdel-Gaber, R. A., 2009. *Ichthyophthirius multifiliis* infection induces apoptosis in different species of Tilapia. *J. Egypt Soc. Parasitol*., 39(2): 665-678

Evans, B. B. &Lester, R. J. G., 2001. Parasites of ornamental fish imported to Australia. *Bull. Eur. Assoc. Fish Pathol*.21: 51–55

Iqbal, Z., Minhas, I. K and Khan, M.N., 2001b. Seasonal occurrence of Lernaeasis in pond Aquaculture in Punjab. *Proc. Pak. Cong. Zool*., 21:159-168.

Iqbal, Z., A. Shafaqat and F. Haroon, 2012a. *Lernaea* diversity and infection in Indian and Chinese carps under semi-intensive culture conditions in Lahore, Punjab. *J. Anim. Plant Sci*, 22(4): 923-926.

Iqbal. Z, Rani I and Pervaiz K, 2012.Treatment of lernaeasis in carps with Thunder, an Organophosphate. *Pak. J. Zool*., Vol. 44(2): 415-420.

Iqbal, Z., Mumtaz, R. and Sajjad, R., 2013. Argulosis, in some Ornamental Fishes Imported to Lahore, Pakistan. *Euro. J. Vet. Medicine.* 2(3): 171-178

Iqbal, Zand Uzma Hussain, 2013. Parasitic infection of an ornamental fish, Shubunkin, *Carassius auratus* L. imported to Pakistan. Biologia (Pakistan),59(2): 211-216

Iqbal, Z., Uzma Hussain, Maryium Abdul Bark and Beenish Gul Rehman, 2013. Incidence of White Spot Disease in Freshwater Ornamental Fishes Imported to Pakistan. Biologia (Pakistan), 59(2): 253-257.

Iqbal, Z Sonia S. Shukerova and Imtiaz K. Minhas, 2014. Occurrence of Black spot diseases in *Labeo rohita* (Hamilton) fry in Carp Hatchery Lahore, Pakistan. Canadian J. Pure and Applied Sciences. 8(1): 2727-2731.

Kim, Jeong-Ho., Hayward, C. J., Joh Seong-Joh. & Heo, Gang Joan, 2002. Parasitic infection in live Fresh water tropical fishes imported to Korea. *Dis. Aquat. Org.,*52: 169-173.

Minhas, I. K., Iqbal, Z. and Naeem Khan.2001.Control *Lernaea cyprinacea* using Dipterex (O, O dimethyl-2-2-2 trichloro-1 hydroxylephosponate) in fishpond in Punjab, Pakistan. *Sci. Int*. (Lahore).13(4), 385-387.

Ogut, H. & Palm, H.W., 2005.Seasonal Dynamics of *Trichodina* spp.on whiting (*Merlangiusmerlangus*) in relation to organic pollution on the eastern Black Coast of Turkey. *Parasitol. Res*. Berlim. 96: 149-153.

Pekmezic, G. Z., B. Yardimi, C.S. Bolukbas, Y.E. Beyhan and S. Umur. 2009. Mortality due to heavy infestation of *Aegulus foliaceus* (Linnaeus, 1758)in pond-reared carp, *Cyprinus carpio* L.1758 (Pisces). *Crustaceana,*84(5-6): 533-537.

Ramudu, K.R., Dash, G and Abraham, T. J., 2013. Parasitic study of *Cirrhinus mrigala* (Hamilton, 1822) in selected districts of West Bengal, India. Int. J. Advanced Biotech. Research. 4 (4): 419-436.

Rasouli, S., A. Nekuifard, D. Azadikhah, H. Ahari, A.A. Anvar, A. Khodadadi and A. Ghasemi, 2011. Ectoparasitic infection of

Carasiuss auratus in water resources of West Azerbijan. Iran. *Iran. J. Fish.Sci*, 4: 156-164.

Raissy, M., Ansari, M., Lashkari, A. & Jalali, B., 2010. Occurrence of parasites in selected fish species in Gandoman Lagoon, Iran. *Iranian J. Fish. Sci*. 9(3): 464-471.

Tonguthai K., 1997. Control of freshwater fish parasites; a Southeast Asian prospective. *Int. J. Para*.,27: 1185-1191.

Thilakaratne, I. D., Rajapaksha, G., Hewakopara, A., Rajapakse, R. P. & Faizal, A.C., 2003.Parasitic infections in freshwater ornamental fish in Sri Lanka. *Dis. Aquat. Org.,* 54:157–162

Ventura, M. T. & Paperna, I., 1985. Histopathology of *Ichthyophthirius multifiliis* infections in Fishes. *J. Fish Biol*, 27:185-203

Other References

Bauer, O., Musselius, V & Stsrelvok, YU, A., 1973. *Disease of Pond fishes.* Israel Program for Scientific Translation, Keter Press Jerusalem.230pp.

Egusa, S., 1982. Infectious Diseases of Fish. Amerind Publishing Co. Pvt. Ltd. New Dehli India.

Hoffman, G.L, 1999. *Parasites of North American Freshwater Fishes.* Cornell University Press, NY.

Hoole, D., B. David, B. Peterand W. Ian, 2001. *Diseases of Carp and other Cyprinid Fishes*. Fishing News Books, Oxford, UK. pp 264.

Inglis, V., Roberts, R.J and Bromage, N. (eds.) 1993. Bacterial Diseaes of Fish. Blackwell Science Ltd. Oxford, UK

Kabata, Z., 1985. *Parasites and Diseases of fish cultured in Tropics*. Taylor and Francis, London. 318pp

Noga, E. J., 2010. Fish Disease, Diagnosis and Treatment.2nd Ed. Wiley-Blackwell. Iowa. USA

Fijan, N., 1976. Diseases of cyprinid in Europe. *Fish Pathology,* 10: 129-134.

Iqbal, Z., Minhas, I. K and Khan, M.N., 2000. Disease Prevalence in culturable fish species in Punjab. *Pakistan J. Fish.,* 1(2):103-112.

Iqbal, Z., Minhas, I. K and Khan, M.N., 2001a. Aquaculture Development in Punjab, Pakistan. A case study. *Sci. Inter*.(Lahore)., 13(1): 59-65 .

Lom, J. & Dykova, I., 1992. *Protozoan Parasites of Fishes*. Development in Aquaculture and Fisheries Science, Elsevier, Amsterdam.253-259pp

Post. G., 1987. Text Book of Fish Health. TFH Publication, Neptune City NJ, 288pp

Roberts H.E, 2010. Fundamentals of Ornamental Fish Health, 1st. Edition. Blackwell Publishers. NY, pp: 28-108

Meyer, F. P. 1991. Aquaculture Diseases and Health management. *Anim. Sci.* 69:4201-4208.

Roberts, R.J., 1989. *Fish Pathology,* 2 nd edition. Bailliore Tindall Publisher, London. pp479

Schaperclaus, W., 1991. Fish Diseases.Vol.1 &2. Amerind Publishing Co. Pvt. Ltd. New Delhi, India

Toor, S.H., Sehgal, H and Sehdev, R.S., 1983. A case study of acute fish diseases in tanks loaded with high level of organic manures. *Aquaculture.*35: 277-282.

Willoughby, L.G., 1994. *Fungi and Fish Diseases.* Pisces Press, Sterling. UK. pp: 57.

Woo, P. T. K., 2006. *Fish Diseases and Disorders.* Vol. 1: Protozoan and Metazoans, 2nd ed., CABI Publication. Oxford. UK. 791pp

CHAPTER – IX

FISH HATCHERY MANAGEMENT

Author

Mr. Iftikhar Ahmad Qureshi

Director, Hatchery Management
Department of Fisheries
Government of Punjab
Lahore – Pakistan
E-mail: iftiaqi@gmail.com

Co-author

Mr. Iftikhar Ahmad

Deputy Director Fisheries
Fish Hatchery Rawalpindi
Department of Fisheries
Government of Punjab
Rawalpindi - Pakistan
E-mail: iftikhargujjar63@gmail.com

The aquaculture industry is continuously expanding the variety of fish species being farmed. Availability of quality seed in adequate quantities is the basic and foremost requirement for sustainable aquaculture. Introduction of artificial breeding techniques and construction of modern hatcheries has helped to meet the demands of the fish culturists. It is estimated that at the current pace of expansion total aquaculture production by 2025 will exceed 60 million metric tons, up from 15 million metric tons in 1990 (Delgado *et al.*, 2003; FAO, 2009). Hatchery operators look for innovations in broodstock management and rearing of spawn to improve quality to ensure desired growth in production and optimum survival. Existing public and private fish hatcheries are playing, and will play, a pivotal role meeting the emerging fish seed demands of the farmers of Pakistan. Recommendations to build and operate a successful fish hatchery follow below.

SELECTION OF SITE

The selection of site is a critical determinant of the success of a hatchery business. The site should be easily accessible and in the vicinity of the farms where fish can be sold at reasonable transportation costs. Site should have access to good quality water available year round. Access to reliable electricity will also be a requirement. In this chapter, we use the model of a carp hatchery for generic purposes. Other species of fish may have minor differences.

WATER QUALITY

Good quality water is fundamental to the success of a fish hatchery. Ideally each fish hatchery has a continuous supply of good quality water at least during its operation for induced spawning and rearing of the eggs, fry and juveniles. During spawning and hatching, oxygen rich water with ambient temperature in the range for spawning is useful for successful accomplishment of this process. Dissolved oxygen level in water can be enhanced by aeration before its entry into the hatchery proper or with aeration or oxygenation in the tanks, but at extra cost.

Underground water is preferable because canal or rainwater can carry a variety of pollutants and harbor predators. The silt in canal water may smother eggs, and adversely affect their developmental process. Rainwater is exceptionally soft with virtually no minerals and likely will cause osmotic stress to eggs, larvae or fry.

LAYOUT PLAN OF HATCHERY

Components of a typical fish hatchery

The hatchery is a composite facility consisting of many essential components which are:

- Broodfish ponds or tanks to grow and hold adult fish who would serve as parent stock for the hatchery, and to accommodate the spent females and males; (Fig.1 & 2)
- An indoor hatchery facility including cement, plastic, or fiberglass tanks for fish spawning, hatching and care of hatchlings to raise them up to post-larval stage; (Fig. 3 & 4)
- Tanks or nursery ponds for rearing post-larvae to fry stage when these are shifted from the indoor hatchery facility; (Fig. 5 & 6)
- Rearing ponds or tanks for growing fry to fingerlings ready for marketing.

Fig.1

Fig.2

Fig.3

Fig.4

Fig.5

Fig.6

Carp Brood fish Requirement

Brood fish is the parent stock from which fish fry and fingerlings are produced. The quality and requisite quantity of fish seed supply depends on health and sufficient number of brood fish. The major carps and Chinese carps generally have high fecundities which vary according to species. Trout and tilapia produce fewer eggs per female. Fecundity is a function of the age of the brood fish, the size, its nutritional state and, therefore, the type of feeds it has been given. For the various carp species, female brood fish weighing 3-6 kg each can be presumed to produce 240,000 viable eggs each spawning cycle. Spawners for the various carp species are paired in male to female ratio of 3:2, keeping the total weight of female brood fish approximately equal to that of male brood fish. One thousand kilograms of brood fish can be comfortably reared in each hectare of fish pond. A brood fish pond of 0.2 ha area is appropriate for convenient netting, feeding, aeration, application of prophylactic and therapeutic chemicals as well as flushing the pond with fresh cool water for proper gonadal development. More brood fish ponds are required for higher fish seed production targets.

TYPES OF PONDS AT THE HATCHERY

The salient features of different types of ponds are:

Broodfish Ponds

A broodfish pond, depending on the magnitude of operations, may vary in area from 0.2 ha to 2.5 ha and, for the sake of ease of netting operations, should be of a rectangular shape, with width not exceeding 25 m. Its water depth may vary from 1.5 m to about 2.5 m. A brood fish pond must be drainable and should be supplied with adequate quantities of organic and inorganic fertilizers for sufficient natural pond productivity and for proper gonadal development of the breeders.

The number of broodfish ponds in a hatchery depends on methods of dealing with different species. The alternatives are: (1) poly-culture of broodfish of both sexes of all the species of Chinese and Indian major carps in the same pond; (2) segregation of a combination of two or more species of both sexes in the same pond; (3) segregation of both sexes of a single species and (4) sex-wise segregation of different species in separate ponds.

Nursery Ponds

They are small in size (0.01-0.1 ha) and are drainable with depth around 0.5 to 1.2 m. Pond bottom should gently slope towards outlet. They are typically rectangular in shape and have a convenient width for netting operations. A harvesting basin is preferred in front of the drain or outside the pond for safe and convenient collection of fry.

Rearing Ponds

Rearing ponds are larger than nursery ponds and are 0.8 to 2 m deep. Pond bottom should be gently sloping towards outlet. They are rectangular in shape and width is far less than commercial units for convenient and on demand netting operations. Like nursery ponds, a harvesting basin is maintained for safe collection of fry and fingerlings.

Drainability of both nursery and rearing ponds is highly desirable because during the slack season, it would enable the pond bottom to be exposed to sun, aeration, mineralization and aerobic oxidation of the organic load which otherwise might lead to hydrogen sulfide production, which is toxic.

Location of Ponds in a Hatchery

The area closest to the hatchery building should be planned for water treatment, (filtration, sedimentation units, aeration tower) laboratory, hatchery office and residential area for the staff. This arrangement will place the nurseries closest to the hatchery proper, which is a logical arrangement. The fish breeding tanks from inside the hatchery building may lead to outdoor nursery ponds by suitable conduits. As hatchlings grow into fry and fingerlings, they can be, with least expense, conveniently transferred from nursery to rearing to stock ponds. According to the aforementioned construction scheme brood fish ponds are the farthest from the hatchery proper hence need proper watch and ward arrangements.

THE HATCHERY PROPER

A modern hatchery is a facility for induced breeding for multiple species of fish. It is a system composed of holding tanks, breeding tanks, hatching jars and larvae rearing tanks. The system of these components can be adjusted as per the commercial requirements of the quantity of fish seed. It has a major role in providing the quality fish seed in required numbers at appropriate times.

The purpose and functioning of different components are described below:

a. Holding tanks

The primary purpose of a holding tank (Fig.4) is to hold ripe breeders before hypophysation, and before spawning, but they also serve other purposes such as holding or even rearing of fry, holding of fingerlings before sale, and providing space for the application of treatments to fish. If the holding tanks are meant as multipurpose in a hatchery, their dimensions may be adjusted as per requirements of the system maintaining their desired attributes and their number.

b. Breeding Tanks

These are used to hold injected or otherwise ripe breeders awaiting induced or natural spawning and fertilization. Fitted with an adjustable stand pipe they have a slope towards central drain for complete drainage of tank water when and where required. The inlet provides uninterrupted flow of oxygenated fresh water.

c. Circular Spawning, Hatchery and Larvae Rearing Tanks

Circular spawning tanks (Fig.3) create a natural environment and facilitate fish spawning. Furthermore, there are no dead areas and the water flow throughout the tank is uniform and water inlet and outlets can be precisely controlled. These circular tanks can serve as breeding tank, hatching tank as well as larval rearing tanks simultaneously.

A convenient size of a breeding tank is 2 m diameter and 1 m deep which would hold about 1,800 liters of water. To maintain water in circular motion, small inlet pipes, upper ones facing the wall and lower ones facing the central drain pipe are installed to keep eggs and fry in suspension with uniform distribution throughout the tank. Normal flow rate in these tanks is 18 L min^{-1}.

CARP BROODFISH SELECTION AND MANAGEMENT

Prior to natural or induced spawning, brood stock should be reared under optimum water quality conditions and suitable types of feed. Formulated feed and/or natural food can be provided to optimize gonadal development. Appropriate and standardized stocking density and species combination ratio is another important parameter to consider for healthy development of gonads (Mirza and Bhatti, 1993).

In addition to formulated and natural feed, application of inorganic and organic fertilizers is also important for maintaining beneficial plankton levels of at least 50 ml per 1,000 L^{-1}. High protein (at least 30%) artificial diet at 2% of their wet biomass is advisable to supplement the natural food in a pond. Catla carp breeding and subsequent rearing is very limited and demands comprehensive research work to resolve its issue of breeding and supply which is always in high demand but unfortunately production is very low.

Reproductive Biology and Induced Breeding of Carps

The Indian major carps and the Chinese carps breed naturally only in the flowing waters of their natural habitat during the rainy season (Mirza *et al.*, 1992). Prior to introduction of induced spawning, the required quantity of seed was collected from natural waters which were frequently contaminated with undesirable species harboring unwanted contaminants and disease causing pathogens. Induced breeding produces seed of much greater consistency and fish can be spawned on demand when it matures. It also provides ample opportunities for stock improvement by selective breeding and hybridization.

Feeding the Broodfish

Special dietary care is required for the different species of broodfish.

***Ctenopharyngodon idella* (Grass carp)**

1. ***Grass Carp:*** In Pakistan, the spawners of grass carp are typically fed once daily, during autumn and spring, on wheat and paddy sprouts, corn grains and bean cakes in equal proportions at 1-2% of their body weights besides macro-vegetation which is fed at 30% of their body weight to enhance fecundity and promote proper gonad development.

2. ***Bighead and Silver Carp:*** These fishes are planktophagous. Fertilization of pond with organic manures at the rate of 1.5 to 2 tons per hectare at an interval of every ten days is recommended. Should the gonad development remain poor, supplementary feeding with powdered bean cakes, peanut cakes and wheat or rice bran may be given in equal proportions at 1-2% of body weight per day may

be given. Formulated pelleted brood stock feeds should also soon be on the market.

Aristichthyhs nobilis **(Bighead carp)**

Hypophthalmicthys molitrix **(Silver carp)**

Catla: For proper gonadal development, organic manuring with cattle dung and/or chicken and duck droppings, and production of zooplankton at 30-50 ml per 1,000 L^{-1} of water are very important. Formulated feeds containing soybean meal and/or fish meal (30% protein) with rice bran or wheat bran with appropriate levels of oilcakes at 3-5% of body weight daily are also valuable.

***Catla catla* (Thaila)**

Mrigal, Rohu and Common Carp: These species are naturally detritus eaters or browsers. Feeds based on soybean meal, rice bran or wheat bran and oilcakes in equal proportions at 3-5% of body weight per day benefit their gonadal development. An alternate feed is a mixture of wheat bran, mustard oilcake, coarse wheat flour and fish meal or soybean meal in the ratios of 4:4:1:1. Mustard oilcake, if used, should be presoaked (for softening) in water overnight.

***Labeo rohita* (Rohu)**

***Cirrhinus mrigala* (Mori)**

***Cyprinus carpio* (Common carp)**

BROOD STOCK SELECTION AND MAINTENANCE

Factors influencing breeding in fishes

1. *Temperature:* Cloudy and rainy weather (temperature range of 24^0C to 31^0C) is good for the stimulation of breeding events in carps. Different events in natural and induced breeding of carps are:

2. *Environmental stimuli* → Receptors → Brain Centre (Hypothalamus) → Pituitary gland → Gonadotrophin hormone → Gonads → Spawning/Natural Breeding

3. *Inducing agents*: During the course of induced spawning of these carps, Carp Pituitary Homogenate (CPH) or some synthetic hormones (gonadotropin analogues) can be used to stimulate early maturation of gonads and spawning.

Stages of Maturation of Adult Carps

Although the development of eggs in the gonads and maturation of eggs is a continuous process, it can be divided into various stages of development and maturation. Once the ovaries have become opaque, granular and somewhat grayish, they occupy about one third of the body cavity of the female. At this stage, the abdomen of the female fish shows a conspicuous bulge which extends past the pelvis up to genital aperture. Further development of the ovary is very fast, especially in increasing day-length and rising temperature.

While sexes in these carps are morphologically similar, they do exhibit some seasonal sexual dimorphism. The more important external distinguishing features of ripe female and male Chinese carps and Indian major carps are that the pectoral fin of male is relatively long and prominent with well-developed thick outer-most ray with a rough inner surface.

The reproductive cycle of the major carps can be divided into following four periods.

i.	Preparatory Period	February – March
ii.	Pre-spawning period	April – May
iii.	Spawning Period	June – August
iv.	Post Spawning Period	September - January

The periods mentioned above are variable according to the environmental condition especially the air and water temperature in the area. Pre-spawning period is associated with the increasing photoperiod and spawning phase is associated with environmental factors prevailing during the monsoon season. Spawning phase includes maturation and ovulation. In induced breeding operation, the purpose of injecting gonadotropin as fish pituitary gland or its synthetic analogue is to help in maturation and ovulation.

Selecting the Broodfish for Spawning

The morphological features described above can be taken as indicators of ripeness. However, a more reliable method of determining whether or not the female fish is ready for spawning is to collect an egg sample from the posterior end of the ovary by means of a catheter. A catheter is a glass tube of 2 mm diameter with a short flexible tubing attached to one end to permit easy movement. The catheter is inserted into the genital aperture and probed to reach the posterior extremity of the ovary. An egg sample is then withdrawn into the glass tube and transferred to a glass bowl into which a solution of 70% acetic acid and 30% alcohol is immediately poured, immersing the ovarian ova sample. Within about 5 minutes, yolk becomes discernible and so also the nuclei of individual ova. A centric or peripheral location of nuclei is a sure indication of readiness of the fish for spawning (Jhingran, 1985). In gametogenesis, the nucleus of the ovum migrates to the periphery before release of the first polar body in meiosis. Should, however, the ova nuclei be found to be centrally located, then such a fish will not respond to hormonal manipulation.

Induced Spawning of Chinese and Indian Major Carps

The most commonly adopted technique of induced breeding of carps is hypophysation which involves injecting mature female and male carps with extracts of pituitary gland taken from other mature fish generally phylogenetically close to carps (Bhatti *et al.*, 1992; Mirza *et al.*, 1992). The pituitary gland (hypophysis) is an endocrine gland located on the ventral side of the brain of the fish. In order to prepare fresh extract of pituitary, the gland is removed by exposing the dorsal side of the head of the fish. If the gland is not to be used immediately for preparation of an extract for fish breeding, it may be preserved. Recently the use of Carp Pituitary Homogenate has been replaced with Ovaprim-C which contains LHRH-a and domperidone, a dopamine inhibitor. The use of Ovaprim-C has revolutionized the process of induced spawning of Chinese and major carps. It can be used in a single dose of 0.5 ml/ kg in the case of female fish and 0.1 ml/Kg for male fish (Naik and Mirza, 1992).

Injecting the Brooders

For the induced spawning of carps, mature male and female brooders are selected based on external secondary sexual characters. The selected fish are transported to an indoor hatchery facility and kept in holding tanks for at least 12 hours for conditioning. This not only helps the broodfish to become acclimated to the indoor environmental conditions but also helps to defecate the gut contents of the fish and get rid of fecal matter that may contaminate the eggs. After the conditioning, the female brooders to be injected are reexamined for their maturity level and transferred to the circular breeding tanks.

Induced Breeding of Grass carp

Induced Breeding of Silver carp

Females are injected intramuscularly with Ovaprim in a single dose of 0.5 – 0.70 ml/Kg body weight, while males are given 0.1– 0.20 ml/Kg of body weight. The males are released with the injected females. The injection dosages vary with stage of fish maturity and water temperature, a relatively lower stage of maturity and cooler water require higher dosages.

Intramuscular injections are generally given at the base of the dorsal fin and /or above the lateral line on the caudal fin, at the base of pectoral fin or between the anal fin and lateral line. A fish scale should be lifted for inserting the injection needle, and the area should be gently massaged following the withdrawal of the needle after injection to aid distribution of the solvent into musculature and prevent its backflow.

Induced Breeding of *Catla catla* (Thaila)

In fish farms and hatcheries where artificially controlled environmental conditions are not available, induced spawning work should be done on cloudy or rainy days. The injection is generally given late in the evening. Such timings generally suit the convenience of workers and the diurnal temperatures are also the lowest when spawning condition of the injected fish is expected (early in the morning). These considerations of timing and weather are of vital importance, especially where breeding and hatching tanks are located in the open, as otherwise the mid-morning sun could cause the water of concrete breeding and hatching tanks to heat up and destroy the ovulated eggs totally. Injected broodfish of Chinese and Indian major carps are released in breeding tanks in appropriately paired sets. Generally, a spawning is made up of one female fish with two males of equal weight. Spawning and fertilization generally take place naturally within 8-12 hours after injection depending upon prevailing water temperature.

Dry stripping

A soft belly of the female at any part of the abdomen, and a state of shifting softness towards posterior indicates readiness of female for stripping. In this condition slight pressure on abdomen expresses out loosened eggs from the genital aperture. For stripping, the female fish is held by the operator between the side of his body and his arm with the fish slanting head up, tail down and belly facing the vessel and the eggs are collected into a plastic trough by pressing the body of the fish. The process is repeated with males as milt is squeezed out into the same basin as the eggs. Two persons can conveniently manage stripping, with one holding the fish firmly and the other pressing the belly for release of eggs with simultaneous spraying of milt on egg mass. At least three persons are required for simultaneous release of eggs and milt. Eggs are then mixed with milt on them with a clean bird feather to allow fertilization to take place. The fertilized eggs are then washed a few times with clean water to remove excess milt and allowed to stay undisturbed in fresh water for about 30 minutes. The eggs are then hardened and are ready for release in the hatching tanks.

Hatching and Care of Hatchlings

Naturally spawned and fertilized eggs of carps, if allowed to hatch in stagnant ponds are often exposed to heavy predation and infestation by pathogens and/or to sub-optimal environmental conditions which considerably reduces percent hatching. For this reason, fertilized eggs are generally collected after water-hardening and then transferred to special incubating circular tanks for hatching. Hatching and the care of hatchlings and larval rearing, in fact, constitute the most important aspects of hatchery management and draw a line between success and failure of the operations. The circular hatching tanks are supplied with oxygenated water of the right temperature and a flow rate of 12-18 liters/minute is maintained in the flow-through system.

After the eggs are hatched, the hatchlings are kept in the tanks for about three days, during which period they need no exogenous food since they subsist on their own yolk sacs. It normally takes slightly more than three days for the yolk sac to be absorbed. After yolk sac absorption their mouths develop and they are ready to take exogenous food.

Fry Rearing In Nursery Tanks

Preparation and maintenance of nursery, rearing and stock ponds are important steps in carp hatchery operations. Carp post larvae and fry are delicate and if transferred to poor food and high predator ponds will lead to virtual decimation of the stock. The purpose of nursery, rearing and stock pond preparation before stocking and maintenance after stocking is not only to remove the causes of poor survival, growth and health of the stocked material but also to optimize good husbandry factors for rearing the young of the species concerned.

Larval rearing in a carp hatchery is carried out in two phases. The first phase is the rearing of post larvae to fry stage which is carried out in nursery ponds and the second stage is the rearing of fry to the fingerling stage which is carried out in rearing ponds. In exceptional cases, these steps are combined in one pond. However, instead of rearing post larvae to fingerlings in the same pond continuously over three to four months, it is a better practice to break it up into two operations in two different types of water bodies, nursery and rearing ponds, as stated above. This is because there are differences between the post larvae and fry in their food, stocking rates and environmental requirements as well as it will discourage accumulation of predators and obnoxious organic matter at the bottom.

The next step comprises adoption of the measures in the sequence stated and at timings specified in the following account of nursery pond management. These measures fall into three categories:

1. Steps to be taken before stocking post larvae in the nursery ponds;

2. Procedures to be adopted during stocking;

3. Steps to be taken after stocking until the production of fry and their harvesting either for sale or for further rearing into fingerlings in rearing ponds.

Pre-Stocking Practices

For preparing the nursery ponds for post larvae in nursery tanks, they should be dried and ploughed to get rid of excess organic matter present in the pond bottom. If the nursery pond cannot be dried, then some suitable pesticide should be used to kill the existing fish stock and other harmful organisms present in the ponds. This is especially true to reduce the risk of predation and cannibalism. For thorough disinfection of a pond, a dose of 1,000 kg/ha of quicklime is required, but if liming has been regularly done annually in the past, 100-200 kg/ha are enough unless the pond soil is very acidic or very poor in carbonates. Quicklime alone can be used in place of a toxicant to kill wild fish, insects and tadpoles and additionally bestowing on the pond the other benefits of liming. For this purpose, a dose of 900 to 1,050 kg/ha of quicklime is necessary if there is little water in the pond. If the pond is full of water up to the brim, the dose of quicklime should be increased to 1,575 to 2,250 kg/ha.

Successive treatments with quicklime and suitable pesticide give most effective results in killing unwanted pond biota, including predatory and weed fish as well as in giving the benefits of liming.

The advantages of liming a pond are numerous. In general, liming enhances pond productivity and improves its sanitation. It is both prophylactic and therapeutic. Specific advantages are that it kills bacteria, fish parasites and their intermediate life-history stages; hence, especially efficacious in a pond where there has been outbreak of an infectious disease. Secondly it builds up alkaline reserve and effectively stops fluctuations of pH by its buffering action which renders acidic waters usable for aquaculture by raising their pH to alkaline levels; and promoting mineralization. The commonly available and used forms of lime are calcium carbonate (ground limestone), calcium hydroxide (slaked lime) and calcium oxide (quicklime). Calcium carbonate dissolves slowly and is of special value for pond fertilization and building up alkaline reserve also leading to calcium enrichment. Quicklime rapidly binds acids and influences pH rapidly, producing results similar to other forms of lime in half the quantity. Lime can be applied to the pond bottom, added to water at inlets or uniformly broadcast on the water surface depending on the form of lime used and the purpose of application. Calcium hydroxide and calcium oxide are best applied on the pond bottom after it has been drained.

Then ponds are fertilized with organic and inorganic fertilizers for sustained production of adequate quantities of zooplankton which form the natural food of carp hatchlings and fry. In the early days of carp culture, the production of any species of zooplankton, whether a cladoceran, a copepod, a rotifer or a protozoan, regardless of size, was considered adequate in nursery ponds. However, in recent times it has become clear that the post larvae of carps survive and grow best if they are fed on smaller planktonic forms like free-living protozoa and rotifers; and that fry and fingerlings, whose mouths are bigger than those of post larvae, grow best if fed on larger planktonic organisms like cladocerans and copepods. In fact, it has long been known that some species of copepods prey on carp larvae and post larvae.

For production of zooplankton, nursery ponds are treated either with organic manures (such as cattle, pig or chicken manure) alone and/or with inorganic fertilizers such as NPK mixtures. If both organic manures and inorganic fertilizers are used they may be applied either one, following the other or as a mixture. If animal manure is to be used alone, its dose should depend on the fish toxicant used for the eradication of unwanted fishes. The cattle manure should be applied at an initial dose of 10,000-15,000 kg/ha about a week before the anticipated date of stocking followed by further manuring at the rate of 5,000 kg/ha seven days after stocking. These manuring rates produce enough zooplankton for a single crop of post larvae stocked at 1.5 million/ha. If two or more crops of fry are required from the same nursery pond, then the pond should be fertilized with 2,000 kg/ha of cattle dung about a week before each subsequent stocking. If poultry manure is to be added instead of cattle manure, one-third the dose of the cattle waste is sufficient since it is at least three times richer in nitrogen, phosphorous, potassium and calcium than cattle manure.

If organic manures are not available, then commercial compost, as used for agriculture, may be applied as a substitute. The recommended dose for compost is 5,000 kg/ha two weeks before the anticipated date of stocking, followed by 5,000 kg/ha a week after stocking. If, however, compost is to be produced in the carp hatchery itself, then the procedure to adopt is to dig the requisite number of pits about 4 m x 3 m x 2.5 m deep at an isolated location and dump green vegetation in heaps about 30 cm high, alternating with 7.5 cm high layers of cattle manure, both dusted liberally with calcium superphosphate. A ratio of 10:1 carbon to nitrogen is required for rapid decomposition of vegetable matter in composting. For this purpose, 25 kg sodium nitrate (per 1,000 kg of compost) should be applied with 4,000 liters of water to provide the necessary humidity in the compost pits. The compost should be turned initially three weeks after dumping and thereafter after every five weeks again. Compost may be expected to be ready in a total period of about 12 weeks after filling the pit at temperatures ranging from 18°C to 25^{0}C.

A mixture of organic and inorganic manures applied at the following rates has given good results: cattle manure at the rate of 20,000-25,000 kg/ha per year and inorganic fertilizer mix (ammonium sulphate + single superphosphate + calcium nitrate in the ratio 11:5:1) at the rate of 1,380-1,725 kg/ha per year applied in 4-10 equal installments is spread over the year.

Stocking Carp Post larvae in Nursery Ponds

After controlling all factors which might lead to mortality of the stocked carp post larvae, as described in the preceding section, the most appropriate time for stocking a nursery pond is when it abounds with zooplankton, especially rotifers and cladocerans in adequate density (preferably rotifers only). Before actually stocking the nursery pond with post larvae, it is necessary to make an estimate of the types and abundance of plankton present. This can be done by adopting appropriate limnological methods. A rough field method for estimating plankton has been developed in which about 55 liters of water, taken from different sections of the nursery pond, are filtered through an organdy or muslin ring net with a 2.5 cm diameter glass specimen tube tied to the lower end of the net. A pinch of powdered common salt is added to the water in the tube after the plankton is collected and the tube detached from the net. Within 15-20 minutes of adding the salt, most of the organisms settle on the bottom. If the column of plankton sediment is at least 15 mm high from the bottom of the tube and the sediment found to consist mostly of cladocerans and rotifers, the pond may be considered sufficiently rich in plankton to stock at the rate of 1.5 million post larvae per hectare. The animal or plant nature of plankton sediment is roughly indicated by either a pale-brownish or greenish color of the sediment, the former indicating preponderance of zooplankton and the latter of phytoplankton. If the predominant plankton population be not of zooplankton, then further organic manuring should be done at doses recommended earlier to rectify the situation before stocking.

To avoid any abrupt change in quality and temperature between the water of the hatching tank and that of the nursery pond, the post larvae should be kept in a suitable container having water initially from the former (hatchery tank) to which the water from the nursery pond should be gradually added in stages, eventually substituting almost the entire hatchery water of the container by the water from the nursery pond. The container should then be slowly dipped and tilted in the nursery pond letting the larvae free to swim out of the container. Stocking should be done late in the evening or early morning, a procedure which gives post- larvae time to acclimatize themselves during the ensuing night relatively free from the predations of enemies. Stocking rate of post larvae in a nursery pond depends on the management practices being attended. If natural food in the form of zooplankton is to be produced by continued pond manuring and supplemental feeds are also to be given, and if facilities exist to remedy oxygen deficiency condition should this occur under conditions of heavy stocking, then the stocking rate may be as high as 10 million per hectare.

Post Stocking Practices

Feeding is based on natural live food organisms generated in the pond itself and augmented through fertilization and on supplemental feeds given exogenously. There is a great deal of difference between complete fish feeds and supplemental feeds. Soon after being stocked in manured nursery ponds containing rich zooplankton, carp post larvae start grazing voraciously on natural food. At this time the feed requirements of spawn are so huge that within two to three days of stocking, the plankton initially present in the pond gets exhausted and steps must be taken not only to generate more natural food but also to administer artificial feeds. Supplementary feeding with manuring of a pond, when done simultaneously, leads to high survival and fast growth of the stocked post larvae in nurseries. The commonly administered artificial feeds for common carp, Chinese and Indian major carps are rice bran and oilcakes of ground nut, coconut, mustard, soybean meal, etc. Artificial feeds typically refer to various ingredients which are mixed together and broadcast on the surface of the water. Formulated feeds are feeds which mix finely ground ingredients together using a standard formula then extruded or pelleted. For young fish, the resulting pellets may be crushed or even reground, to make a crumble or powder. Artificial and formulated feeds are always given in finely powdered form to carp post larvae. Formulated feeds are more likely to contain significant amounts of soybean meal and oil due to the highly nutritious characteristics, ease of use, price and consistent availability.

It has been found that among the combinations of various artificial and formulated feeds containing protein, fat, carbohydrate, minerals, roughage and vitamins, etc., the maximum growth of post larvae is obtainable with feeds having a combination of hydrolyzed proteins and carbohydrates (50:30). Complex proteins and pure carbohydrates give poorer results. Further, rice bran alone, a food most often given singly, gives much poorer results than a mixture of oilcakes, rice powder and black gram in powdered form. Silkworm pupae, soy, and fishmeal give still better results.

A feed compounded from dried and finely powdered and sieved notonectids (which are highly predatory to carp spawn), small prawns and shrimps and cheap pulses or lentils in the ratio of 5:3:2 gives much better results for survival and growth of hatchlings of

Catla, rohu, mrigal and silver carp than the conventional mixture of rice bran and oilcake, besides utilizing an otherwise wasted product.

For monoculture of rohu spawn, good results are obtainable with zooplankton, followed by silkworm pupae, mustard oilcake with rice bran and ground nut cake with wheat bran in the order stated. Normally post larvae stocked in nursery ponds attain a length 2.0 to 2.5 cm (when they are termed fry) in about 15 days with artificial feeding giving a survival of about 50% in earthen ponds. A higher survival rate may be expected if a system of intensive controlled culture of rotifers and crustaceans to serve as fry food is developed and facilities for intensive aeration are provided. The fry may be transferred to prepared fry-rearing tanks or otherwise taken care of for further rearing if not sold to the fish farmers.

Rearing Pond Management

Rearing or growout ponds are deeper and larger than nursery ponds, and are more liable to get infested with weeds. An overgrowth of weeds deprives the pond soil of nutritive elements, restricts the movement of fish, interferes with netting operations and harbors predatory and weed fishes and insects. Weeds occupying different habitats and niches have to be controlled in different ways. Floating weeds like *Eichhornia* and *Pistia* are best removed by manual labor. Chemical herbicides like 2, 4-D are quite effective and economical against *Eichhornia,* though not as much against *Pistia.* When mixed with common domestic detergent, 2, 4-D acts effectively against weeds like *Pistia, Nymphaea* and *Nelumbo* in which leaves are either hairy or waxy. Simazine WP-50, another herbicide, applied at 5.6-11.2 kg/ha kills *Pistia, Eichhornia* and *Colocasia* completely within two to three weeks even during rains. Taficide-80, a third herbicide, at a dose of 2.2 kg/ha, is also effective against *Eichhornia.* Marginal weeds like *Typha,* grasses, sedges, rushes, *Ipomoea, Jussiae, Jagittaria* and *Cocasia* are effectively controlled by ploughing in, grazing by livestock, burning during dry season or repeated cutting and deepening or marginal shelves. All herbicides should only be used strictly according to the directions on the manufacturer's label. In fact, in Pakistan and most other countries it is illegal to use the herbicide in any manner other than the labeled directions. Workers applying herbicides should be provided with proper protective clothing and safety gear including respirators. Applicators should also receive training in proper use of the gear, explicit training in methods of application according to the label and first aid in case of accident.

Rooted emergent weeds like *Limnanthemum, Trapa, Myriophyllum,* etc. are successfully removed by repeated cutting of leaves before fruiting at weekly intervals, for about six to eight weeks. Alternatively, spraying once or twice with, 2, 4-D (at 5.6-11.2 kg/ha) kills these plants. Rooted submerged weeds are cleared by a number of simple, manually operated devices like bottom rakes, log weeders, metal spikes, with or without barbed wire attachment, forks, drag chains or bamboo poles fixed with cross pieces at their lower ends followed by repeated netting with strong wire or rope nets. Other methods employed include shading by floating plants like *Pistia* or *Salvinia* for a period of 8-10 weeks or by creating algal blooms or algal mats by repeated fertilization with N:P:K fertilizers.

The best known fish that is used for biological control of weeds is grass carp, *Ctenopharyngodon idella.* Grass carp feeds most voraciously on *Hydrilla, Najas* and *Ceratophyllum.* It can also control infestations of *Ottelia, Vallisneria, Nechmandra, Utricularia, Trapa, Myriophyllum* and *Limnophila.* Anhydrous ammonia gas, obtainable in gas cylinders, controls *Hydrilla, Najas, Wolffia, Nymphaea, Ottelia, Limnanthemum* and *Nelumbo* when injected in the subsurface layers with an applicator at 112-334 kg/ha or 6.9-19 ppm. Much of the ammonia applied enters the pond's production cycle by nitrogen enrichment resulting in phenomenal growth of plankton soon after weed clearance. Some blue-green algae, particularly, *Microcystis,* can form long persistent blooms which deplete oxygen and often cause fish mortality. Simazine at a dose of 0.5 to 1.0 ppm clears algal blooms and mats and brings about prolonged control without affecting production of other plankton and fish.

For further rearing of carp fry to fingerlings, either monoculture or polyculture may be carried out. The stocking density of fry measuring 25.4 mm to 37.8 mm weighing 0.15 to 0.75 g each may be 125,000/ha to 250,000/ha. A practice recommended for healthy fry rearing is that the size of the fry in a rearing pond at the time of stocking should be as uniform as possible. This is accomplished by first segregating the catch in holding pens, made of gunny or canvas bottom and split bamboo sides supported on stakes. It is necessary to periodically clean the holding pen of debris, fish excreta, etc. which is done by lifting the bottom and removing the rubbish manually. Size grading is done by sifting fry through sieves of different mesh gradations made of split bamboo. Fry attain the fingerling length of 100 mm to 172 mm in about three months. A survival of up to 80% may be expected provided that a full complement of management measures for environmental enrichment are adopted and that the fry have natural food as well as artificial feed given to them. Artificial feeding may be on oilcake, mustard or ground nut or coconut and rice bran in the ratio of 1:1. The density of feeding in the first month may be equal to the initial total weight of fry stocked daily and in the second and third months, twice the initial weight of fry stocked daily.

In the case of nursery ponds, harvesting may be done by repeated seining with fine meshed nets. In rearing ponds, where fry are grown to fingerling stage, periodical harvesting may be done at an appropriate time to avoid overcrowding. After harvesting, the fingerlings may be stocked unless sold. The function of a carp hatchery is completed after fingerlings have been produced.

BREEDING OF TILAPIA

Mono-sex Tilapia Seed Production

Tilapia are mouth breeders, and in the genus *Oreochromis*, the female tilapia lay their eggs in pit nests built by a male and after fertilization by the male, the female collects eggs in her mouth (buccal cavity) and keeps there until hatching (Iq and Shu-Chien, 2011). This poses a problem when large numbers of eggs must be collected for rearing of fish seed on commercial basis. To overcome this problem, the tilapia spawning should be synchronized to produce progeny groups as uniform in size and age as possible. Spawning of Tilapias is influenced by both environmental (e.g. photoperiod, temperature, food availability) and social factors (social stimuli exchanged between neighboring females) (Costa-Pierce, 1997). Therefore, the strategy for synchronizing spawning involves maintaining brood stock separated by sex in a suitable holding facility, conditioning by proper feeding, and evaluation of the sexual maturity condition of females. Tilapia brood stocks are readily conditioned with good feeding, warming water temperatures, and separation of the sexes for a short while. In higher latitudes, warming water temperatures with lengthening photoperiods will further condition the fish.

***Oreochromis niloticus* (Nile Tilapia)**

***Oreochromis* spp. (Red hybrid tilapia)**

Breeding in Hapas

A 'hapa' is a fixed net enclosure, similar to a mosquito net. It is typically made of polyethylene netting sewn into an open top cage with nylon thread. Hapas of different sizes can be used but the most commonly used size is 1 m (width) x 2 m (length) x 1 m (depth) with a mesh size of 1.0 to 2.0 mm. Several males will be kept in one hapa and females in another. Holding potential male and female breeders together helps to synchronize spawning by exchanging social stimuli (e.g. pheromones) (Huertas et al., 2014).

After conditioning, the female breeders should be checked for their readiness to spawn by visually examining their morphological characteristics. Female breeders are then categorized in one of the following maturity conditions: 'ready to spawn' (RS), 'swollen' (S), 'not ready to spawn' (NRS), and 'has spawned' (HS). Female breeders categorized as 'ready to spawn' are first selected for pairing with a male in breeding hapas.

Stocking the Breeders

The female breeders are stocked into the breeding hapas before the males. The ripe males are then transferred to the hapas with females that are most ready to spawn, with a stocking density of one male and three females per hapa. After fry are produced and collected, the males are separated from the female and returned to the male holding hapa.

Fry Collection and Incubation

The fry are normally collected early in the morning to reduce stress and mortalities. The first fry collection is initiated 10 to 14 days after stocking the breeders. For this purpose, a pole is placed under the hapa and lifted in order to divide the hapa into two compartments with the breeders all on one side. The male is removed and returned to the male holding hapa. Then the workers proceed to checking each of the females. Females with incubating eggs or young yolk sac fry in their mouth can be placed into the empty portion of the breeding hapa until yolk absorption is complete or until the fry are in the free-swimming stage. Free swimming fry or advanced yolk sac fry are rinsed out of the mouth of the mother into a tray or bucket. Eggs or yolk sac fry that have been accidentally released from the mouth of the female can be collected and transferred to artificial incubators. Both fry and eggs are rinsed and counted before transferring them to the nursery hapas or artificial incubators.

It is important to ensure a constant flow-through of water to the incubators to optimize the environment for the eggs or yolk sac fry. Upwelling incubator bottles are most commonly used. Trays are the second most common. Upwelling bottles provide better aeration and motion, while trays are more convenient for observation and removal of unfertilized eggs and dead embryos, both of which encourage fungal growth. The eggs usually hatch after 2 or 3 days. The fry are incubated until yolk absorption is complete and are then transferred to nursery hapas.

Tilapia hatching in trays

Tilapia hatching in upwelling bottles

Under FEEDing Pakistan project, with technical support of ASA/WISHH/SoyPak, first tilapia hatchery is established in private sector at Tawakkal fish farms in Muzaffargarh. Photo courtesy, R.S.N. Janjua, SoyPak (ASA/WISHH)

Pedigree Breeding Program and Marker Assisted Breeding

Genetically improved fish, such as the Genetically Improved Farmed Tilapia (GIFT) and Chitralada strains, have become popular for culture in developing countries (Wijenayake et al., 2005; Tan et al., 2011). These are fast growing strains that have been developed by genetic selection of desirable characteristics over successive generations. A continuous improvement of relevant traits over the generations requires a well-designed selective breeding program where the pedigree of brood fish is monitored to increase the accuracy of selection and to restrict inbreeding.

SEED HANDLING AND TRANSPORTING

Transporting Live Fish

One of the most frequent activities on any fish farm is the moving of fish around from one tank to another, or from pond to pond, or even the collection of broodstock from elsewhere. Sorting stock, stocking grow-out tanks, moving broodstock, harvesting fingerlings, sampling growth rates, and catching adult fish for selling are all examples of why someone needs to move fish. The following points need to be considered when moving fish. :

i. Crowded fish will soon die of oxygen depletion in buckets or small containers.

ii. Fish transferred to water of different temperature, pH, hardness or alkalinity, will need an acclimation period. The greater the changes, the longer the acclimation period.

iii. Transferred fish experience stress by catching them and confining them under crowded conditions. Stress makes them more susceptible to disease.

Short Distances

When moving fish short distances within the hatchery itself, they can be transported in buckets. Remember that certain fish almost invariably try to jump out of buckets so the buckets need lids or netting covers. Fish that jump out of buckets and fall on the ground are easily injured, lose their protective coating of slime, and may die within a few days of stocking.

Long Distances

Special protocols should be implemented when moving fish over longer distances, such as when stocking ponds with fingerlings or obtaining broodstock. These fish should not be fed for at least 24 hours (preferably 48 hours with tilapia, due to their long gut length) before transporting them as they will foul the hauling water and poison themselves and other fishes. To purge or 'starve' fish prior to packaging and transporting they should be starved in clean, algae-free water in containers or tanks like plastic or concrete pools, with clean running water. This also allows sorting into different sizes for safe packing and transportation.

Packing Small Fish Or Fingerlings

Small fish (1-5 cm) can be packed in plastic bags placed in cardboard cartons for safe transportation. Hauling water should be of the same temperature from which fish were collected and should be rich with oxygen.

It is not advisable to pack fish using water from the rearing container which may contain elevated ammonia and/or low oxygen levels. It is preferable to use fresh

and clean well water. Plastic bags should be a minimum 40 micron thickness for small fish and preferably 60-80 microns for fish like tilapia with sharp spines. One third of the plastic bag is filled with water. Required number of fish is transferred into it and the bag is inflated with oxygen. A half gram of Tris buffer can be added to maintain the pH level and a few pieces of activated carbon to absorb ammonia and small organic compounds. The neck of the bag is twisted several turns and then tightly tied with non-flexible string, strong rubber bands, or rubber castration rings. The inner bag is then placed inside an outer bag. Many people prefer to layer a couple of sheets of newspaper between the inner and outer bag to reduce chance of a fish spine puncturing both bags. The newspaper also darkens the environment for the fish, provides extra insulation and absorbs any water that may leak from small punctures. It is important to check the bottom of the bag to prevent small fish becoming trapped in the corners. This is essential and an often overlooked procedure; if a few fish become trapped in the corners and die, they will rapidly decompose in warm weather and poison many of the others. The bags are then placed within a cardboard box, bucket or polystyrene box to prevent them being punctured. The polystyrene boxes will help insulate the fish from rapid temperature changes.

When transporting the boxes they should not be placed in the back of an open truck, exposed to the sun or cold, as the small volumes of water will rapidly either overheat or cool down, so a canopy is essential. On arrival at the destination the fish will be considerably stressed, and the bags should be carefully floated in the water for 15 minutes or more to equalize the temperature differences. Then the bags should be opened and kept floating by rolling the bag down to create a float. Receiving water should be added into the bag water for another five or ten minute to acclimate pH, hardness and alkalinity. Once this is done the bags can be tipped over and the fish are released.

Packing Large Fish

For large fish over 10-12 cm, plastic bags puncture too easily and other containers should be utilized. For small numbers of fish, Styrofoam or insulated coolers are good for transport. For larger numbers of fish, plastic 50-200 liter drums with a top that can be secured are useful. For short time intervals (<15 minutes) large fish can be moved around the farm in these drums without oxygenation, if not over-packed (not more than 5-10 adult fish per 100 liter drum). If the fish are likely to be in the drum for longer periods, portable battery-operated air-blowers or bottled compressed oxygen can be used to oxygenate the water using an airline and air stone in each drum. Drums should be filled to about 30% full when packing the fish, then lifted into the vehicle, and then topped up to 80% full with clean water to prevent too much sloshing and damage to the fish on rough roads. All this should be done under shade to prevent the water warming up to the point where oxygen levels deplete and fish may die due to suffocation. If hauling distance is long (2 hours or more), then provide oxygen to each container. A small air pump working off the vehicle battery is adequate for small biomasses of fish. Compressed oxygen bubbled through the water is essential for trout or if the densities or total biomass are very high.

At the destination, the drums should be first partially emptied using buckets, then the drums lifted down and the fish poured slowly into the dam, pond or other container. If the receiving water is different to the drum-water temperature or pH, then some of this water should be added to the drum, water slowly, over half an hour, to equalize temperatures and pH. While this is being done, the flow of air or oxygen to the tanks should be kept operating.

Packing Un-Purged Fish Taken From a Dam Or Pond

It is unwise to pack fish that have been caught from ponds or tanks where they have recently fed to satiation. This is because they have a gut full of food that will be expelled in the packing water, thus polluting it. Tilapia are especially difficult in this respect due to their long gut length and their continuous eating habits. Tilapia caught and packed directly after capture will quickly foul the water. Only short journeys for unpurged fish are possible without oxygen or in some way purging the fish prior to packing. One solution is to hold them in a portable plastic pool, at the pond-side in the shade, with clean water, for some hours after netting them to attempt to purge them of most of the gut contents. Use of one or more portable air-blowers can assist in keeping this holding water well oxygenated. If purging is not possible, the maximum packing density recommended for carp and tilapia is no more than 6-10 adult fish per 100 liter drum for travel that is not more than 4-6 hours. Aeration will be essential.

Size-Sorting the Fish

After the fish have been collected in buckets, they should be sorted by sizes or species. If sorting is done quickly, small fish can be returned to the pond to allow them to grow further. If size-sorting is needed, it is recommended that one or more portable fish pools be erected in a shady area near the pond to be harvested. All undersized fish can be immediately placed in these pools to rid them of the mud and other plant debris that invariably clogs their gills and fins while being netted. If large quantities of fish are to be caught, a flow of fresh water from a pipe may be required to keep the water in the portable pools. Fish may die quickly if hauled in warm or muddy water.

References

Bhatti, M. Z., I. U. Naik and Z. S. Mirza, 1992. Induced spawning of Grass Carp (*Ctenopharyngodon idella*) using carp pituitary homogenate and human chorionic gonadotropin in Punjab, Pakistan. *Proc. Cong. Zool.,* 12: 429-433.

Costa-Pierce, B. A. R. J. W. A. S. A. T. A., 1997. *Tilapia aquaculture in the Americas*. Baton Rouge, La.; [S.l.]: World Aquaculture Society ; American Tilapia Association.

Delgado, C., N. Wada, M. Rosegrant, S. Meijer and M. Ahmed. 2003. Fish to 2020: Supply and demand in changing global markets. WorldFish Center Technical Report 62: Intl Food Policy Research Inst.

FAO, 2009. *The state of world fisheries and aquaculture: 2008*. Rome: Food and Agriculture Organization of the United Nations (FAO).

FAO, 2012. *The state of world fisheries and aquaculture : 2012*. Rome: Food and Agriculture Organization of the United Nations (FAO).

Fernandes, A., É. Alvarenga, D. Oliveira, C. Aleixo, S. Prado, R. Luz, N. Sarmento, E. Teixeira, M. Luz and E. Turra, 2013.

Production of Oocytes of Nile Tilapia (*Oreochromis niloticus*) for In Vitro Fertilization via Hormonal Treatments. *Reprod. Domest. Anim.,* 48 (6): 1049-1055.

Huertas, M., O. G. Almeida, A. V. Canário and P. C. Hubbard. 2014. Tilapia male urinary pheromone stimulates female reproductive axis. *Gen. Comp. Endocrinol.,* 196: 106-111.

IQ, K. C. and A. C. Shu-Chien. 2011. Proteomics of buccal cavity mucus in female tilapia fish (*Oreochromis spp.*): a comparison between parental and non-parental fish. *PLoS ONE,* 6 (4): e18555.

Jhingran, V. G. P. R. S. V., 1985. *A hatchery manual for the common, Chinese, and Indian major carps*. Manila, Philippines; Portland, OR: Asian Development Bank : International Center for Living Aquatic Resources Management; Exclusive distributor, ISBS.

Mirza, M. R. and M. N. Bhatti, 1993. *Fish and fisheries of Pakistan, Part I*. Lahore: Ferozsons.

Mirza, Z. S., I. U. Naik and M. Z. Bhatti. 1992. Induced spawning of Indian Major Carps using carp pituitary homogenate and human chorionic gonadotropin in Punjab (Pakistan). *Proc. Cong. Zool.,* 12: 423-428.

Naik, I. U. and Z. S. Mirza. 1992. Use of Ovaprim-C in induced spawning of Indian Major Carps in Punjab, Pakistan. *Proc. Cong. Zool.,* 12: 411-416.

Tan, R., Y. Garcia, M. Dator, I. Tan and D. Pemsl, 2011. Technical efficiency of Genetically Improved Farmed Tilapia (GIFT) cage culture operations in the lakes of Laguna and Batangas, Philippines. *Journal of ISSAAS (International Society for Southeast Asian Agricultural Sciences),* 17 (1): 194-207.

Wijenayake, W. M. H. K., U. A. D. Jayasinghe, U. S. Amarasinghe, J. A. Athula, K. B. C. Pushpalatha and S. S. DE Silva. 2005. Culture-based fisheries in non-perennial reservoirs in Sri Lanka: production and relative performance of stocked species. *Fish. Manag. Ecol.,* 12 (4): 249-258.

CHAPTER – X

ENTERPRISE MANAGEMENT

Author
Dr. Kevin Fitzsimmons, Ph.D.
Professor of Soil, Water and Environmental Science
Director for International Agriculture Programs
1140 E. South campus Drive
Forbes 306, University of Arizona
Tucson, AZ 85719, U.S.A.

A. BUSINESS PLANS

Preparing a business plan is the single most important step in starting an aquaculture venture. A business plan should address each of the technical aspects described in each of the chapters of this handbook as well as a detailed description of the expected markets for the products of the aquaculture operation. The market for what the farm produces is the single most important business aspect. A business plan should clearly identify the specific product that will produced, who else will compete with you for this market, and the prices that you expect to receive for the product compared to your expected costs to provide this product. The business plan should also provide a sensitivity analysis describing what would happen to the business if key costs (feed, labor, utilities) should increase by certain amounts or if key buyers or prices were to decrease (sales drops of 10 or 20%). Most importantly the business plan should demonstrate some key commitments from potential buyers. It should be noted that letters of interest from potential buyers are nice to have, but that they cannot be depended upon as firm commitments. Investors, banks and other lenders will want these in a business plan but they realize that buyers will rarely sign a firm commitment for purchase of product at a set price before a project is started. The buyers will almost always be pleased to have potential new suppliers enter a market.

The next most important aspect of the business plan will be the financial projections. The financials should include a detailed description of the all the capital expenditure costs to get the farm built and the operational costs that will carry the business forward. Having sufficient capital and operating funds to carry the business forward through the time that sales income consistently exceed the operating costs is critical and is frequently overlooked or underestimated. This is probably the single most common cause of business failures. Operating costs frequently increase more rapidly than sales income for new firms. This has been especially true for aquaculture operations as costs are tied to large international markets for feed ingredients (soy, wheat, corn, and fish meal), utilities, and labor, while fish sales must compete with wild caught fish which go up and down in price and with competing new aquaculture ventures.

A business plan should also incorporate critical financial issues including interest rates, loan origination fees, management fees, insurance, municipal, state and federal taxes, depreciation rates and schedules, rental fees, and alternative opportunities for funds, equipment, labor and land devoted to the business. These are all important to the final decision to pursue a particular investment or project.

B. ENTERPRISE BUDGET

An enterprise budget is a valuable shortened version of a business plan. In an enterprise budget the project is usually considered for a single year or crop. The intent is to examine all the input costs and sales income that can be expected from that particular crop. An enterprise budget may be determined for a particular pond, crop, or farm. This will allow the farmer to examine a simplified version of the potential business.

a. Input costs

In a typical enterprise budget the input costs will include feed, labor, fuel, utilities, chemicals, fingerlings, rented equipment, services, advertising, and other expenses directed related to that particular crop. The businessman farmer will be using this tool as a way to determine if it is advisable to proceed with a potential venture. If it is obvious that the expenses will exceed the costs of production, the rest of the financial aspects will almost certainly fail.

b. Sales

The income from expected sales should be a conservative number based on historic prices obtained in the same markets into which a farmer will be selling, preferably, for the same product, sold in the same outlets, to the same consumers in recent times. It is easy for farmers to exaggerate the anticipated prices they expect to receive, especially when they are bringing new volumes of product into a market. Additional supply will almost always depress product prices unless demand is increased in some way. Advertising may help to increase demand and thus prices and income, but the advertising costs will then need to be added to input costs. Again, if sales income in an enterprise budget does not exceed the input costs, there is little reason to proceed as the basics of the business will be probably be undesirable.

c. Income-Expenditure Sheets

Book keeping in an old fashioned ledger-book is always a good idea in addition to more sophisticated business software. A careful recording of all costs and income on a daily basis is necessary for the farm owner or manager to understand the fundamentals of his or her aquaculture business. Business software with spreadsheet programs and/or professional accounting and tax advice are necessary for virtually any business now, but simple accounting procedures as part of an enterprise budget are always advisable so that basic operations of the farm operation are not overlooked. All of the data from a ledger, double entry, or account book will need to be entered into a software program eventually, but having an on-farm hard copy collected by staff that can be referred to in order to confirm expenditures and income is the best way to avoid clerical errors.

C. PERSONNEL (Human Resources and Record Keeping)

Staff members of an aquaculture operation are critical to the eventual success or failure. In general, farm staff members with agricultural backgrounds, especially with animal husbandry, have proven to adapt to rearing fish with minimal effort. Fishermen have a less successful record, although some have done fine especially with marine caged fish. Livestock farmers often have a better understanding of the need for constant attention to the needs of the fish. They are also often better at judging feeding levels, reaching satiation and developing a feel for when the fish are not in optimal condition.

At any size operation, the human resources of the farm are as important as the fish resources. The staff members should feel that the welfare of the fish and the enterprise will be related to their benefits and that the success of the farm will improve their welfare as well, whether in salary bonuses, improved housing or diet. It is important to follow all legal requirements for employment and social welfare and corporate responsibilities. Increasingly, international buyers are requiring that these conditions are met through certification programs.

Record keeping of farm operations is critical to successful operations. In addition to the financial records described above, farm operating records are also important. Proper recording of amounts fed, fish feeding activity and feed inventory are important to control the single greatest cost of an aquaculture operation. Monitoring of water conditions (temperatures, dissolved oxygen, ammonia, nitrite, nitrates, pH, and alkalinity) is critical to maintaining the proper environment for the fish and ensuring that healthy conditions will allow for fast growth. Careful recording of growth rates and any mortalities and general condition and activity of the fish is necessary to ensure a high survival rate and to predict any disease or pathogen issues when they are easy to deal with and before they can become catastrophic. Even if the farmer does not recognize a developing health concern, a disease expert will need this information and records of the changes in order to diagnose a problem and recommend a course of action. With no records, a pathologist or epidemiologist will need to take time to conduct a series of tests that will delay any treatments or farm operational adjustments.

D. REGULATORY MANAGEMENT (Farming licenses, Environmental regulations, Food safety)

Pakistan has a series of rules and regulations pertaining to aquaculture, water pumping and use, and general farming activities. Each aquaculture farmer is responsible for ensuring that all legal requirements are met and that licenses and permits are obtained and kept current. There are also a series of environmental rules and regulations regarding which species of fish can be farmed in different parts of the country, how waters are discharged from farms and which chemicals and antibiotics can be used legally in the proper manner. Finally, there are regulations pertaining to food safety and the safe handling of animals bound for human consumption. The farmer and his or her processing and handling partners are responsible for not only providing safe seafood products but also meeting the regulatory guidelines provided by the government. In Pakistan, several parasites infecting humans, with birds, fish, and/or snails as vectors are known to exist. Most farms attempt to exclude birds from the production systems as likely predators as well as potential vectors of fish and human parasites and pathogens. Snails are likewise considered both an annoyance to equipment as well as potential vectors. Predator control and snail control are both portions of an overall farm biosecurity program. Biosecurity and the wider ranging Best Management Practices (BMPs) also seek to reduce contamination of fish from environmental pollutants. BMPs or Best Aquaculture Practices (BAPs) include planning for how to reduce or avoid pollutants entering the farm through the water, air, feed, or even on the workers. Best management practices will be described in greater detail below. Some of these rules and regulations are mentioned in this handbook, however, this book is not meant to be a definitive list and the aquaculturist must be responsible for contacting Pakistani government representatives to ensure full compliance.

E. CERTIFICATION PROGRAMS

National governments, industry cooperatives, non-governmental organizations and the Food and Agriculture Organization of the United Nations (UN-FAO) have all determined that certification programs are useful to differentiate the most responsible growers and products from the farms with the weaker producers. Best management practices or best aquaculture practices have been codified by several groups including the UN FAO, the Chinese and Thai national governments and a host of non-governmental organizations, food retailers, and other institutions (Aquaculture Stewardship Council, Aquaculture Certification Council, Natural Land, Global Good Agriculture Practices, WalMart, Whole Foods, and Monterey Bay Aquarium). In each case the certification program intends to reward the producer and/or the processor for implementing activities that provide a safe and productive workplace that is fair to workers, meets environmental safeguards and provides safe and healthy seafood to consumers. Each of the certifying organizations operates by reviewing sustainability of aquaculture practices and providing a certification and marketing logo that can be affixed to the product packaging, or at announcements at point of sale. The Monterey Bay Aquarium in the U.S. develops a list that is widely circulated with small "watch cards", posters for use at seafood counters and an extensive website.

Certification programs that indicate product and processing compliance with voluntary guidelines, national government regulations, or international standards have grown rapidly and have expanded to include farm operations, hatcheries, feed mills and processing plants. By working up and down the value chain, the programs seek to provide vertically integrated supervision. It was activity working back up the value chain from shrimp farms, to feed mills, to source of fish meal, back to the fishing fleet that discovered the problems with forced labor and mistreatment of immigrant fishers in Thailand in 2013. Proper use of drugs and chemicals is another focal point of certification. Historically, misuse of antibiotics in the salmon industry was a prime focus of certification programs. The wide application of vaccinations for fish has reduced antibiotic use in aquaculture by two orders of magnitude in recent years. Disinfection of nets and other equipment is an important part of the programs and is considered in BMPs and BAPs to protect both the aquatic animals and workers.

The International Standards Organization (ISO) has published guidelines for food processing plants that most seafood processors strive to meet. Most international importers and retailers of seafood require these certifications be in place. ISO 9100 provides for certification of Hazard Analysis at Critical Control Points(HACCP) which covers product safety, plant and food hygiene, economic integrity, and product quality. ISO 22000, the food safety management system, applies to all kinds of food processors linked to Codex Alimentarius.

Hazard Analysis at Critical Control Points (HACCP)

HACCP is a concept in which the human visual inspection of animals being processed into food is replaced with an integrated program which looks at the points in the process which are most likely to contribute to contamination or adulteration of processed foods. The U.S. Food and Drug Administration adopted regulations in 1995 mandating application of HACCP principles for safe and sanitary processing of seafood. By focusing on these critical points with documented record keeping, the processor and the inspectors can be more efficient and improve food safety. HACCP is a planning procedure for documenting good production and processing practices. Participants operate under an approved plan with audits at random frequency. These plans help guide the operations in processing plants and back to the production of the fish. Focus is on documentation of proper activities at important stages rather than stationing a permanent inspector at farm or processing plant. Personal hygiene at the processor is evident at most modern plants which include multiple hand washing, clean uniforms, hair and face coverings, boot washes, and gloves. Many processors now also include a chlorination or ozonated water dip for fillets before packaging. Ozonated water dips especially have been found to reduce surface bacterial counts on fillets (Kim et al., 1999; Restaino *et al*., 1995).

Other examples of parts of a HACCP plan:

1. Document feed source and use, farm water quality, testing for off-flavor

2. Document source, arrival time, temperature and condition of fish as they arrive at process plant
3. Provide footbaths, hand washes and protective clothing for processing workers, document usage by having employees sign daily log
4. Measure and record bacterial numbers on fillets during quality control

The first step is to write the plan. This is typically done with a consultant or experienced government advisor. The second step is plan review by authorities. These are normally from a health inspection agency from a national or regional government. The third step is to train all employees in HACCP plan procedures and documentation. This is a critical aspect as the farm and processor want to ensure product quality, but also to have the staff able to gather the data and complete the required paperwork. The fourth step is to operate the farm and/or plant according to the approved plan. Again the supervisors and the working staff must understand the plan, be capable of conducting tests and collecting data and recording them correctly. Fifth step is to maintain paperwork documenting all stages until inspection. A perfectly run farm or plant that fails to document the facts, will fail the inspection and all that entails (Cato, 1998; Miget, 2004).

F. SUSTAINABLE AQUACULTURE

Operating aquaculture farms in a sustainable manner is the goal of every farmer and investor. Sustainability entails environmental, social and economic aspects as the operation must achieve all three to be successful. Today there are a plethora of descriptions: corporate social responsibility, triple bottom line, win-win-win, etc., that each point to the need to address all aspects of aquaculture and seafood processing and delivery for operations to be sustainable for an extended period. Consideration of reuse of aquaculture effluents, processing by-products, carbon footprint, food miles, and product life cycle each contribute to the consideration that farmers and processors now must consider. We are lucky that aquaculture as an industry has embraced these topics and researchers and innovative farmers are constantly developing answers and improvements that quickly spread through the industry (Costa-Pierce 2008).

References

Cato, J. C., 1998. Seafood safety: Economics of hazard analysis and critical control point (HACCP) programmes (No. 381). UN Food and Agriculture Organization.

Costa-Pierce, B. A. (Ed.), 2008. Ecological aquaculture: the evolution of the blue revolution. John Wiley & Sons.

Kim, J. G., Yousef, A. E., and Dave, S., 1999. Application of ozone for enhancing the microbiological safety and quality of foods: a review. Journal of Food Protection, 62(9), 1071-1087.

Miget, R. J., 2004. The HACCP seafood program and aquaculture. Southern Regional Aquaculture Center.

Restaino, L., Frampton, E. W., Hemphill, J. B., and Palnikar, P., 1995. Efficacy of ozonated water against various food-related microorganisms. Applied and Environmental Microbiology, 61(9), 3471-3475.

CHAPTER – XI

HARVEST, STORAGE AND PROCESSING

Author
Dr. Kevin Fitzsimmons, Ph.D.
Professor of Soil, Water and Environmental Science
Director for International Agriculture Programs
1140 E. South Campus Drive
Forbes 306, University of Arizona
Tucson, AZ 85719, U.S.A.
E-mail: kevfitz@ag.arizona.edu

A. Pre-Harvest and Harvest

B. Processing and packaging

C. Value chain models

D. Quality Control, and Traceability

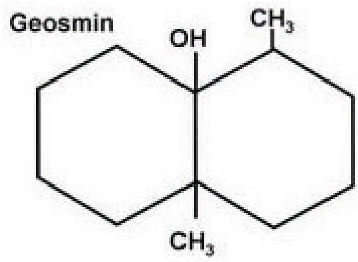
E. Markets and Market Development

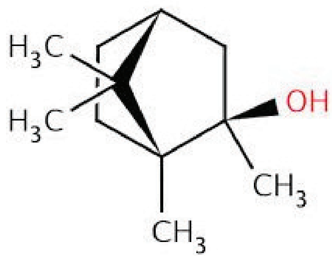
A. PRE-HARVEST AND HARVEST

Quality control of aquaculture products has been one of the most critical aspects to the success of the industry. Maintaining and improving the quality of the various product forms has been a central part of the rapid growth of demand for farmed fish products in the market. This attention to detail starts while the fish are still growing in their various production systems. Processors, haulers and farmers work together to ensure that fish are not contaminated by chemical pollutants, parasites or by spoilage during transport. Virtually all farms check their water sources on a regular basis to ensure high quality. Many farms now use bird nets or greenhouse covers to keep birds and other sources of potential contamination out. In Pakistan, the use of groundwater is one method to maintain quality control and avoid complications that might come with use of canal water or reservoirs.

Off-flavor - The objectionable taste and smells commonly referred to as off-flavor is one of the most important quality factors. Any fish can be affected by off-flavors, but tilapia, carps and catfishes are especially susceptible. Cyanobacteria, also called blue-green algae, are responsible for most off-flavor in farmed fishes. Monitoring for off-flavor is a process that begins before harvest and continues throughout processing. Fish from ponds are most likely to have accumulated the compounds geosmin and/or methyl-isoborneol

Methyl-isoborneol





(MIB), produced by the algae, at levels that would impart objectionable tastes and/or odors. However, intensive recirculating systems have also been known to develop off-flavors. The most common method for determining if fish is off-flavor is to cook a whole fish or a freshly cut fillet in a microwave oven. Most testers will cook the fish or fillet inside a paper bag to concentrate any odors. (Note: plastic bags are not good as they can emit their own odor when heated in a microwave.) The odor may be obvious just by smelling the contents of the bag. Otherwise the taste tester will eat some of the fish to detect off-flavor. Some testers have the ability to detect geosmin and MIB at levels of 4 or 5 parts per billion.

Fish are normally sampled a week before a tentative harvest. Sampling is a relatively simple process. A few fish are harvested from the pond and filleted. The fillets are placed in a small paper bag (lunch bag style). The top is rolled or folded down and placed in a microwave oven. After cooking for thirty to sixty seconds, the bag is opened and the sampler will quickly smell the air in the bag. If the fish has accumulated geosmin or MIB, the sampler should smell it immediately. If the level is low, the sampler may eat a mouthful of the product to confirm. Most people can detect levels of geosmin or MIB at levels of 5 to 10 ppb. Experienced samplers can detect down to levels of 2 or 3 ppb. If detected, the standard method to eliminate off-flavor is to place the fish in clean flowing water for several days. This is normally sufficient to allow for elimination of the offending compounds. Taste testing will be repeated to ensure the fish is free of off-flavors. Most processors will repeat testing at several points in processing as part of their quality control.

Harvest techniques – In Pakistan, harvesting varies considerably depending upon the culture system. Ponds are normally partially drained and then harvested by use of a seine and hand nets or buckets. The fish are typically brought to one side or corner with the seine and then may be lifted out by hand or with a large scoop net or sometimes placed in large bags. The largest farms may utilize a net with a quick release bottom latch suspended from a crane or back-hoe.

Bringing net full of fish to shore

Harvesting fish by hand

More sophisticated ponds and raceways that can be gravity drained may utilize a harvest box that concentrates fish for removal using nets or baskets. Harvest boxes may be built inside the pond or outside in the drain channel.

Cage culture, where fish are reared in net lined enclosures in ponds or lakes, typically uses a large bar placed across the top of the cage to concentrate fish for harvest. One side of the net cage will be pulled up and over the bar, concentrating the fish in the increasingly smaller part of the net. The process continues until the fish are concentrated into one corner where they can be lifted out by hand or scoop net. The largest farms may use fish pumps, fish escalators or other mechanical means to remove fish. Many farms will use graders to separate harvest size fish and either leave small fish in the production system, or remove them to another production unit.

Floating cage culture in large water reservoir

Floating cage culture in canal

Depuration – Depuration is a process designed to improve the quality of fish by allowing the fish to empty the stomach and intestine and leach out any geosmin or MIB by holding the fish in clean water without feeding for one to three days. Depuration has not been commonly used in Pakistan until recently. Most major farms and even some processing plants in other countries now incorporate a depuration stage between harvest and processing. This is normally a specially designed pond or tank system designed to clear the fish of off-flavors and eliminate materials from the gastro-intestinal system. Purging fish in this manner may lead to a 4% loss in weight. This may be a significant additional loss in weight (and income) for the grower, but it greatly reduces the chances of off-flavor, reduces the amount of fish waste in the transport water and reduces the threat of contamination of product with fish waste.

Hauling – In Pakistan, most fish are hauled from the farm to a market on ice. There is no live market for selling fish live to consumers or restaurants and very few farmed fish are sold to processing plants.

In fact as of 2014, 80% of fish were still sold fresh. Most of this fish is also sold in the round (not cut at all). It is important that all fish be chilled as quickly as possible. The very best method is to immerse the fish into an ice water slurry straight from the pond. Insulated plastic totes or ice chests are most effective. Totes or ice chests should be loaded onto a truck or trailer when empty and then filled with equal amounts of ice and water, as water and ice add a tremendous amount of weight. As most farms do not have forklifts or back hoes, this will mostly likely be done by hand. Fish should be harvested from the pond and placed directly into the slurry. The fish should not be bled or eviscerated until they arrive at a processing plant or the final consumer asks for the fish to be cut.

In many countries fish are delivered alive to the processing plant, restaurant, grocery store or live sale shop to assure the highest quality of the product. At larger fully integrated farms, the processing plant may be on-site and fish may be delivered by flume or other mechanical means. When delivered from a remote farm, fish are delivered in live haul trucks. Crude live haulers may utilize an open top canvas bag suspended by rails on a stake-bed truck. More sophisticated haulers use specially designed fish hauling boxes equipped with aerators or bottled oxygen. In all cases it is important to deliver fish to the buyer alive and with a minimum of physical damage. Some haulers will begin chilling fish, but most will deliver fish at ambient temperatures.

Live sales - In Pakistan the sale of live fish is still a novelty. However, in most of Asia this is a regular practice at several levels of marketing. Fish are frequently delivered live to a processing plant, distribution center or directly to a grocery, restaurant or live fish shop. In these cases, the fish should be kept with as little stress as possible and the holding tanks should maintain conditions favorable to the fish. Live tanks usually operate like large aquaria with a biofilter, mechanical filter, recirculation pump, air blower and diffuser in the tank. For the farmer, two great advantages of live sales are the entire fish is sold with no processing waste and the sale can eliminate one or more middle men. Customers who have enjoyed really fresh fish will pay for the extra costs to enjoy product that was alive only minutes before being served.

B. Processing and packaging

Processing and food quality requirements vary considerably from country to country. U.S., European Union, other multinational organizations and several non-governmental organizations (NGO's)

Organization	Pertinent Website
International Standard Organization (ISO)	http://www.iso.org/iso/home/standards/management-standards/iso22000.htm
Food and Agriculture Organization (FAO)	http://www.codexalimentarius.org
Global Good Agriculture Practices (GAP)	http://www.globalgap.org
USDA	http://www.fda.gov/Food/GuidanceRegulation/default.htm
Aquaculture Stewardship Council	http://www.asc-aqua.org
Aquaculture Certification Council	http://www.aquaculturecertification.org

guidelines are continually updated as public health concerns, consumer interests and technology evolve. Hazard Analysis at Critical Control Points (HACCP) http://www.fda.gov/Food/GuidanceRegulation/HACCP/ ucm2006764.htm and other processing guidelines should be examined carefully before deciding on a particular design and operating plan for a processing plant. Likewise, practices vary from plant to plant regarding how and when products are delivered, weighed, scaled, gutted, bled, filleted, how glazes are applied and how product is labeled. Buyers should inspect and agree on product specifics before purchasing.

Processing lines – There are two basic designs to fish processing plants. The first uses a batch process whereby quantities of fish are acted upon at a station and then the product is bunched in totes or baskets and transferred to the next station. The other basic design is a continuous line with product continuing down the line as portions are removed and the final product gets packaged.

Bleeding / chilling – Many processors prefer to bleed fish as a preliminary step. Most often this entails hand cutting the gills of the fish. Some plants will also cut the caudal blood vessels in front of the tail. The intention is to quickly remove much of the blood from the fish, which improves the quality and appearance of the final fillet product. Fish are typically placed in vats of water to bleed. The vats may be at ambient temperature, which will encourage rapid bleeding, or in chilled or iced water, which will begin the chilling process but slow bleeding. Some plants will bleed in ambient water and then add ice to chill in the same vat. Some processors prefer to put newly arrived fish directly into an ice slurry to immediately kill the fish and rapidly chill the carcass. This is more common for fish that will be frozen whole or gutted. It can be counter-productive to chill fish before bleeding is completed, but some processing plans require this in their guidelines.

Scale removal – Some plants use hand labor to remove scales from the carcass while other use mechanical equipment. The most common equipment is a rotating drum with slotted surfaces that tumble the fish to remove scales. Mechanical scrappers are used in many places that use hand filleting without machines. The drum scalers are not used at some plants that deal primarily with hand filleted products.

De-heading – Removal of the head from the carcass is increasingly the standard method at processing plants. This operation can be accomplished using either a food grade band saw, rotating knives mounted in a mechanical de-header and in some cases with a large hand knife or cleaver. Most plants will use either a curved cut or a v-shaped cut in order to recover the flesh behind the head. A few plants still directly remove the fillet from the carcass, leaving the head intact on the skeleton. This was common in plants with an abundance of low cost labor but even these plants are increasing moving toward more automation and recovery of the head as a marketable by-product.

Drum used to remove and rinse off scales

Removing fish head by hand

Evisceration – Removal of the viscera is another common procedure. Typically an incision is made from the anus up to the ventral fins by hand or machine. Some machines may make an incision from where the head has been removed down to the anus. The viscera may be removed by hand, by a high-pressure water jet, or by a suction device. A good depuration system will minimize the amount of undigested feed and fecal material. Again, there are some plants that do not eviscerate as the fillet is taken directly from the carcass.

Fillet – Currently in Pakistan relatively few farmed fish are filleted. However in coming years, this is expected to become much more common. There are several methods of hand filleting. Variations depend on whether the cutter is right or left handed, which side of the fish is being cut and whether the head has already been removed. The type of knife used also varies considerably. Some prefer to use a heavy long shank knife, while others prefer a thin knife, which allows the cutter to easily feel the bones. Others prefer to cut through the rib cage and then remove it as a separate operation. While others leave the rib cage intact and cut around. Most processing plants use a bonus system to reward especially skilled filleters. Typically the bonus is based on the number of fillets that a cutter can recover per time period (hour, shift, pay-period). There are several automated fillet machines that take the entire fish, make several cuts and leave finished fillets. These have not become very popular yet as they need frequent adjustments, trained staff and are very expensive. As they continue to improve and become more reliable, they may start to replace human filleting.

Skinning by hand with pliers

Skinning with fillet knife

Automatic skinner

Skinning – Automatic or mechanical skinners are ubiquitous in the industry. A skin-on fillet is hand fed to the skinner which has rotating rollers that grab the skin and pull it down while the knife edges set on the aperture cut the fillet from the skin. The depth of the cut can be adjusted to leave more or less of the flesh on the fillet. A deeper cut, leaving more of the darker flesh on the skin has become more popular in recent years. A deeper skinning will typically decrease the fillet weight by 5%. New skinners that freeze the skin to a roller and use a movable blade are being tested and may replace the current models. The new skinners leave a smoother cut.

Trimming – The next step is to remove pin bones and trim off the outer edges of the fillet. Normally, several small pin bones that were attached to the ribs are left in the fillet of a tilapia or catfish. In carp, there are many more bones, some of which are not connected to the rest of the skeleton. Thus carp are less popular as a fillet product. For the other fish, typically a v-cut is made to remove them pin bones.

An accomplished trimmer can do this removal with a minimum of waste. The loose supportive tissue along the top of the fillet is often removed, as are thin pieces along the belly portion. These tissues often come off during handling and cooking so the buyers prefer to have it removed during processing. Some plants will also rub the fillet against a roughened plastic surface as a final step to remove any remaining sub-dermal facia.

Trimming tilapia fillets

Typical fillet yields with pin bones removed

Table 1. Fillets yield of different fishes

Fish	Skin on %	Skin off %	Deep skinning and / belly removed %
Rohu carp	51	45	42
Tilapia	35	32	30
Channel catfish	42		27
Rainbow trout	50	45	40
Pangasius	43	38	34

(Memon *et al*, 2011, Fitzsimmons, 2006; Bosworth *et al*., 2001; Testi *et al*., 2006; Nortvedt, 2007.)

Ozone and chlorine baths – Outside of Pakistan, most plants run their trimmed fillets through a water bath at this stage. In the past, some plants used a mild chlorine solution in the water to reduce bacteria and lengthen shelf life. Most plants have now replaced chlorine with ozone gas that is bubbled into the tank. Ozone does not have the disinfection by-products that chlorine does, nor does it leave any disagreeable taste that can be discerned by some consumers. Most plants use an on-site ozone generation system that supplies the small amounts of ozone needed effectively disinfect. Lab studies at the University of Arizona demonstrated that bacterial counts could be lowered by several degrees of magnitude and shelf life could be extended by several days.

Carbon monoxide and liquid smoke – Carbon monoxide (CO) gas has been used in some countries, but apparently not Pakistan, to maintain the appearance of freshness (bright white and red) on the fillet. It appears that the gas is absorbed by the flesh and reacts with myoglobin in the muscle tissue. By binding the myoglobin, fillets maintain a fresh, bright red color in the myomeres for extended periods. In the simplest method, carbon monoxide gas is applied by placing fillets on a tray, which is placed into a large plastic bag. The bag is inflated with gas, tied off and allowed to absorb for 5 to 10 minutes. This method exposed workers to large amounts of carbon monoxide which can cause health problems with extended exposures. An alternate method is to place the trays of fillets into a large cabinet that is filled with the gas. This still exposed workers to CO as the cabinets were opened. Eventually a safer method was developed placing fillets into a retort vessel with high pressure to infuse the CO quickly. The gas could also be evacuated from the vessel and replaced with air before the removal and refilling stage. Several countries do not allow the treatment of fish fillets with carbon monoxide and will not accept imports that have been so treated. The U.S. has reviewed the practice and requires labeling or notification of the procedure on the packaging. Many of the major buyers of fillet products in the U.S. will not accept fillets that have been treated with carbon monoxide. Liquid smoke is a product which is sometimes used to impart a smoked flavor to fish rather than going through the actual smoking process. Smoke from a wood fire is condensed, often with a small amount of water sprayed into the condensing tube and chilled air. The condensed liquid is then is sprayed or injected on a fillet to impart the smoked flavoring. Small amounts of carcinogenic compounds may be present in the liquid.

Freezing – Rapid freezing of the fillet or whole fish is critical to maintain the product quality. Fillets are normally placed on large trays that ride on a conveyer through a tunnel freezer. Often the fish are given a quick dip or hand-sprayed with water to form a glaze over the fillets. This avoids freezer burn (and adds weight). The percent glaze on a fillet is a constant topic of negotiation on the value chain. Glaze extends freezer life by reducing oxidation and freezer burn (drying), but also obviously adds weight, which someone is paying for, even though it is just water that melts away. Whole or gutted fish may go through a tunnel freezer or a blast freezer. Seafood processors in Pakistan have this capability but so far are purchasing very little fish from farmers.

Trimmed tilapia fillets

Individually quick frozen fillets ready for packing

Packaging – Packaging is still in its infancy in Pakistan as is branding and marketing. However, some growers and retailers have begun branding and packaging with marks on boxes and labels. This can be expected to increase as stakeholders attempt to differentiate their products from competitors. When most of the fish in domestic trade are sold whole there is little to differentiate, however as fish are treated better than the average and especially with more processing, the packaging and branding will be more and more important. In the early stages of international trade whole or gutted, product frequently are often transported in large containers holding hundreds of individually quick frozen (IQF) fish. This might be an initial format for selling carps and/or tilapia into Gulf countries. The next typical processing and packaging format might be simply filleting the fish. For domestic markets these might be sold fresh, while for international trade they more likely would be IQF. These would be placed onto individual styrofoam trays with plastic wrap for retail sales. Today, with more sophisticated processing in the producing countries, virtually any style of packaging is available. Many fillets and even whole fish are now packed into individual bags that are heat-sealed or vacuum packed. The bags are normally put into a five or ten pound cardboard or plastic box. These boxes may be placed into an insulated master pack. Fresh fillets are normally packaged in five or ten pound plastic packs that can be resealed, and are preferred by the restaurant trade.

For **tilapia,** the fillets themselves are normally graded by size. Most common grades are 3 oz and under, 3-5 oz, 4-6 oz, 5-7 oz, 6-8 oz, 7-9 oz, and over 9 oz . Many plants have automatic sorting machines that separate fillets by size. In developing countries hand sorting is common and highly accurate with scales used only for checking. The variety of fillet and value added products continue to grow with size variations, skinning variations and various treatments available. Breaded and marinated preparations are the most popular, but new stuffed, baked, broiled and grilled products appear on a regular basis.

Multi-function machines – There are several automated fillet machines that are capable of accepting a whole fish at one end and discharging finished fillets at the other. Many processors feel that the machines are still not cost effective, primarily because they do not recover as much as hand filleting and cannot compete with the low labor costs in most of the major producing countries. Additional innovations should eventually close the gap. There are also several machines that will conduct one or more processing functions. These are also finding their way into processing plants around the world as even the lowest cost labor markets move more toward automation.

C. Value chain models

In Pakistan the majority of carp farmers sell their fish to haulers who then bring the fish into urban brokerages or distribution locations. The prices paid to the farmers by the haulers in April 2014 were in the range of US$ 1.60 to $2.40 per kilo. The variation is due to quality of fish, average size of the fish, and distance from the market. The distributors/brokers will pay the fish haulers from $2.00 to $3.00 per kg. The distributors/brokers will then charge the small retailers US$2.60 to $3:60 per kg for the product delivered to the small shops in urban areas who in many cases have limited space and refrigeration. Consumers for the most part purchase whole fish for US4.00 to $6.00 per kg, which they prepare at home, or have the fish seller head and gut the fish at the store or stand.

Carp Value chain as of April 2014

Farmers - US$1.60-$2.40/kg - Fish Haulers - $2.00-$3.00/kg - Distributor/Broker - $2.60-$3.60 / kg - Small retailer / Grocery - $4.00-$6.00/kg - Consumer

Trout are mostly farmed in the Swat Valley, while the major market for the fish is in the Islamabad/Rawalpindi area. In 2012-2014 most of the farmers have made arrangements to deliver fish fresh on ice directly to retail seafood stores. So the value chain is shorted with farmers being paid by the stores. This minimizes the role of middlemen, but takes time, effort and resources from the farm. The store then sells to the consumer for $8.00 to $12:00 per kg.

Trout Value Chain as of April 2014

Farmers (delivery by farm) -US$5.00 - $6.60 /kg - Small retailer / Grocery - $8.00-12.00/kg – Consumer

Picture. Trout caught at M. Rasheed farm in Madyan, Swat (Photo Courtesy: R.S.N. Janjua, SoyPak (ASA/WISHH) dated 09-11-2013

Tilapia are widely farmed across Punjab and Sindh with markets for the fish in most of the urban areas. In 2012-2014 most of the farmers have made arrangements to deliver fish fresh on ice directly to retail seafood and grocery stores. And in one case the farmer developed his own retail outlet in Karachi. These direct connections may not continue as additional farms add tilapia production to carp ponds. We anticipate that custom harvesters and/or haulers will begin to develop competitive advantages and

Tilapia Value Chain as of April 2014

Farmers (delivery by farm) - US$1.80 - $3.00 /kg - Small retailer/Grocery -$3.00-$5.00 kg - Consumer

Byproducts – Skins have become the most valuable byproduct from processed tilapia (Fitzsimmons, 2006). There are four primary markets. First, skins have been used to make a variety of leather goods. In Brazil, Colombia and Thailand, several companies have extensive product lines including clothing and accessories. The second market is as a snack food. De-scaled skins can be cut into thin strips and deep fried. These are especially popular in Thailand and the Philippines. A third market for skins is as a pharmaceutical product. European companies are substituting material from tilapia skins for mammalian products for gelatin used to make time released medicines. The fourth market for skins is for collagen. In China especially, the skins are processed to recover collagen which is utilized in a large number of beauty care products.

Fish scales are used to make ornamental flowers. Individual scales are collected, and dyed different colors, then carefully glued onto a stick to mimic various flowers.

Tilapia skins dyed for use as leather.

Tilapia scales dyed to make flowers

Another byproduct is the trimmings and heads. Heads are used for soups in some countries. Post-ocular and throat muscles can be recovered and used for ceviche and other preparations using small amounts of meat. Recovery of flesh through deboning of pin bone cuts and skeletons can provide a base for fish sticks or other highly processed forms. Carcasses, heads and trimmings can be used for animal feeds, especially hogs.

D. Quality Control and Traceability

For both domestic and international markets quality control and traceability have become critical factors.

Quality control beyond off-flavor will incorporate visual inspection for parasitic worms, checking for metal contamination during processing, bacterial contamination and chemical pollutants. Visual inspection should be conducted at all stages of the value chain. Metal contamination is both visual and in a processing plant with a metal detection device which will find even small amounts of metal chips or shavings that might come from malfunctioning processing equipment. Bacterial and chemical pollutants will need to be checked with a more sophisticated quality assurance lab with appropriate testing equipment.

Traceability refers to the ability of stakeholders to follow the provenance or chain of custody of the fish from farm to hauler to processor to shipper/exporter to importer to wholesale to retail to the customer. With today's computer technology and organized logistics, bar codes, Quick Response Codes (QR mark), Radio-frequency identification (RFID) tags, real time websites and cloud computing, anyone on the chain can check lot numbers and follow fish products in transit, or on arrival, or retroactively find the origin and history of a shipment or even individual package. There are several companies whose primary focus is to provide these services across a complete value chain and numerous owners of an individual lot. Wisefish http://www.wisefish.com/ is one example. Seafood Traceability: A Practical Guide for the U.S. Industry by Arni Petersen and David Green, is an 30 page document available free on line

http://seafood.oregonstate.edu/.pdf%20Links/Seafood%20Traceability%20-%20A%20Practical%20Guide.pdf that provides and very complete description of tools and software.

E. Markets and Market Development

Pangasius processing for Pakistani markets – Vietnamese catfish, or *Pangasius*, are one of the most popular fillet products in Pakistani grocery stores. Farmers and processors in VietNam have developed a one million metric ton industry in little more than 15years with exports to scores of countries. Virtually all of these fish are grown in intensive ponds with high densities of these air breathing fish. The fish are harvested and delivered live to state of the art processing plants built in recent years. The fillets for international trade are filleted by hand and then individually quick frozen. These are the typical product forms sold in Pakistan.

Tilapia processing for international markets - Taiwan was the first growing area to produce and export significant quantities of tilapia in the 1980's. Most of the exports were whole or gutted frozen fish sent to the U.S. Jamaica was the second major exporter on the world market, sending fresh and frozen fillets to the U.S. and Europe. Just a year or two later, Indonesia began processing cage reared tilapia from reservoirs and exporting frozen fillets. At about the same time, Colombia and Costa Rica began processing fish grown in raceways and semi-intensive ponds and exporting fresh fillets to the U.S. After a series of major disease outbreaks, several shrimp farmers in Ecuador switched to tilapia production. Using existing production, processing and marketing channels, Ecuadorian farms have taken a significant share of the fresh fillet market in the U.S. Using technology and investment from Taiwan, provinces on the mainland of China have become major producers and exporters. Large quantities of frozen fillets are now exported to the US and Europe. Production in Zimbabwe is based on cage operations in Lake Kariba. Fillets from the processing plant are marketed in Europe. Brazil and Thailand, major producers who have sophisticated processing plants but are minimal exporters, have had little impact on the international markets for processed tilapia. Mexico and the Philippines each have major producers who expect to develop international quality processing plants and products in the near future.

The market for fresh fillets has grown to be the most valuable sector. While sales of whole frozen fish have stagnated and frozen fillets have grown steadily, sales of fresh fillets have exploded globally. In virtually every region of the U.S., fresh fillets are available in stores and restaurants. Since 2010, tilapia has been the fourth most commonly consumed seafood in the U.S.

Potential markets for Pakistan – The Pakistani domestic market should be the most profitable for Pakistani trout, tilapia, carp or catfish. There seems to be pent up demand for high quality fresh and locally produced frozen fillets. Outside Pakistan, potential customers include the Gulf Emirates and Saudi Arabia. The third country nationals there are familiar with these fishes and are likely customers. Pakistan has a well-developed food export industry to the Gulf region. The primary competition for frozen fillets of tilapia and carp products would be from the current Egyptian and Chinese suppliers. With the proximity and frequent air connections to the Gulf Cooperation Council countries, the market for fresh fillets would seem to be a more preferable and profitable market niche.

References

Bosworth, B. G., Holland, M., and Brazil, B. L., 2001. Evaluation of ultrasound imagery and body shape to predict carcass and fillet yield in farm-raised catfish. Journal of animal science, 79(6): 1483-1490.

Fitzsimmons, K., 2006. Harvest, Handling, and Processing. pp. 607-618. In: Lim, C and Webster, C., eds. Tilapia: Biology, Culture, and Nutrition. Hawthorn Press.

Memon, N. N., Talpur, F. N., Bhanger, M. I., and Balouch, A., 2011. Changes in fatty acid composition in muscle of three farmed carp fish species (*Labeo rohita, Cirrhinus mrigala, Catla catla*) raised under the same conditions. Food Chemistry, 126(2), 405-410.

Nortvedt, R., 2007. The opportunities in the catfish surplus market in Vietnam. ISBN, 978(82), 7251.

Testi, S., Bonaldo, A., Gatta, P. P., and Badiani, A., 2006. Nutritional traits of dorsal and ventral fillets from three farmed fish species. Food Chemistry, 98(1), 104-111.

CHAPTER – XII

FISH MARKETING AND ECONOMICS

Author

Dr. R. S. N. Janjua

Country Representative
American Soybean Association
World Initiative for Soy in Human Health
Karachi-Pakistan
E-mail: janjua@soypak.org

Co-author

Prof. Dr. Muhammad Ashraf

Dean, Faculty of Fisheries and Wildlife
University of Veterinary and Animal Sciences (UVAS)
Pattoki Campus, Punjab – Pakistan
E-mail: muhammad.ashraf@uvas.edu.pk

INTRODUCTION

Pakistan is endowed with a rich fishery potential. Located in the northern part of the Arabian Sea, it has a coast line of 1,120 km with a broad continental shelf and its Exclusive Economic Zone (EEZ) extends up to 200 nautical miles from the coast. Fisheries and the fishing industry play a significant part in the national economy and as a foreign exchange earning about US$300 million in 2013 (Marine Fisheries Department of Pakistan).

In Pakistan, marine capture fisheries contribute the major share of more than 65% (660,141 MT) of all fisheries production (952,735 MT) and remaining 35% (292,594 MT) comes from inland waters (Source: Marine Fisheries Department). The fisheries sector plays a considerable role in employment opportunities, food security and poverty alleviation in the country. Presently fisheries sector is contributing 1% of the total GDP of the country (Source: Economic Survey of Pakistan 2013). In 2005, imports of fish were negligible but in recent years it has been substantially increased. In addition, trash fish (non-edible for human beings) used for production of fish meal and other species are exported in different forms including salted dried, frozen and chilled forms.

The major quantity of fish produced is consumed locally. While there is a considerable increasing trend in fish consumption due to changing consumer behavior, it is still 2 kg per capita which is far behind the recommended consumption criteria (i.e., 16 kg per annum that the FAO has proffered for health and nutrition reasons). To achieve the desired consumption level, considerable work needs to be done to change consumer behavior particularly the shift from seasonal to non-seasonal catch and consumption. Statistics on inland fish production and survey of fish consumption have not been properly collected and maintained. Thus the fish producer does not know how much he has to produce to cater to the demand of the consumer. Pakistan has a seasonal consumer demand driven market. Another challenge is that the extension services to individual farmers normally focus on improving production, while neglecting marketing, processing (cold chains, live marketing, smoking and value added products), socio-economic factors, and the adoption of aquaculture. The present situation strongly demands an introduction of strong marketing techniques and defined supply chain as it has been developed in poultry and dairy. Most fish producers are not fully aware of market strategies and have been exploited by the middlemen.

FISH MARKETING STATUS IN PAKISTAN

In Pakistan, besides inadequate marketing systems for fish products, there is also a lack of a modern reliable supply chain system. Non-availability of value added, branded products and lack of consumer education are other constraints that hinder the proper development of marketing systems.

Presently on the marketing side there are wide seasonal price fluctuations which lead to uncertain ties in obtaining favorable prices upon harvest. Other problems are delayed payments to fish farmers by wholesalers and commission agents, relatively high fish hauling charges and lack of knowledge about the status of marketing conditions at the time of sales of fish. Lack of adequate information about the status of supply and demand and poor linkage among producers and the wholesaler is another contributing factor in securing prices above the cost of production. There are several reasons for this insecurity, the main ones are seasonal harvest and more specifically perishable product that create a marketing competition between farmers. The farmer is then bound to sell his crop at the will of wholesalers. Usually, the peak season of fish consumption is started from the month of September to April, while May, July, and August are off season or closed season for fish harvesting.

Traditionally there are seven major fish species cultured locally as well as marketed accordingly. These are rohu (*Labeo rohita*), mori (*Cirrhinus mrigala*), thaila also known as catla, (*Catla catla*), grass carp (*Ctenopharyngodon idella*), silver carp (*Hypophthalmichthys molitrix*), bighead carp (*Aristichthys nobilis*) and gulfam (*Cyprinus carpio*). Mossambique tilapia (*Oreochromis mossambicus*) was introduced in the 1970's and it had very slow market growth due to its prolific breeding habits and farmers did not adopt this species as a commercial product. Accordingly, it could not maintain a place in the market because of a preference for bigger sized fish of aforementioned species in markets of Pakistan.

Under the "Feeding Pakistan" project from 2012, the introduction of sex reversed (GIFT) tilapia (*Oreochromis niloticus*) and soy-based extruded floating feeds, attracted several progressive farmers who harvested culture of this species on commercial scale. This species of tilapia grows faster than the Mossambique tilapia and attains the size of 500 g to 900 g in six to eight months after stocking in a well-managed farm. It is hardy and can be farmed under high stocking densities with much higher production per acre than the conventional carp farming being practiced in Pakistan. This size is very acceptable in the market and both intermediaries and consumers prefer its purchase and sales. It has better taste and lacks intramuscular bones (Y-bones) as compared with the farmed carp species. This lack of extra bones seems to drive the consumer preference over the other traditional inland fish species available in the market. Therefore, we need to identify the market intermediaries involved in the marketing of inland fish, their marketing margins and the market channels through which the inland fish reaches the ultimate consumers.

In the light of aforementioned evidence, it is critical to study the markets the farmers must focus on for the following points.

1. Are markets accessible?
2. What have market prices been over the last 5 years?
3. Are they higher during certain months than others?
4. Is the most common market price high enough to cover all production costs?

MAJOR FISH MARKETING CHANNELS IN PAKISTAN

Fresh fish is traded throughout Pakistan but the majority of trading is practiced in Punjab, due to a major percentage of population of Pakistan is resident in Punjab. Retail markets are established in almost all the major cities and towns of all provinces of Pakistan.

Wholesale and distribution markets are limited in the cities and big towns. Marketing of the fish continues throughout the year except June, July and August. Lahore, Rawalpindi, Gujranwala, Faisalabad, Multan, Kasur and Sahiwal are the major fish markets in Punjab. Karachi, Hyderabad, Thatta and Sukker are the major markets in Sindh and there is limited wholesale marketing in Peshawar, KPK and Quetta, Balochistan.

The perishable nature of fish, lack of appropriate transport facilities, day to day fluctuation of fish catch, functioning of un-regulated markets, non-availability of adequate storage facilities and limited taste for fish are the factors which have complicated the marketing system of fish.

FISH MARKETING CHANNELS

The sequence of stages involved in transferring produce from the farm to the consumer is generally referred to as a marketing channel (Shepherd, 1996). The marketing of fish in Pakistan is mostly carried out through indirect channels before it reaches the ultimate consumer. Direct marketing is when there is a direct contact between the producer and consumer. In most of the cases, the producer sells their catch through intermediaries particularly when the consumer markets are distant from the production areas. The common practice of channeling the catch is through the commission agents because the producer has to focus on farm management for better production.

Figure 1 explains the traditional various channels and routes which ultimately collect the fish from the producer and supply it to the consumer covering several steps in between two production methods in Pakistan.

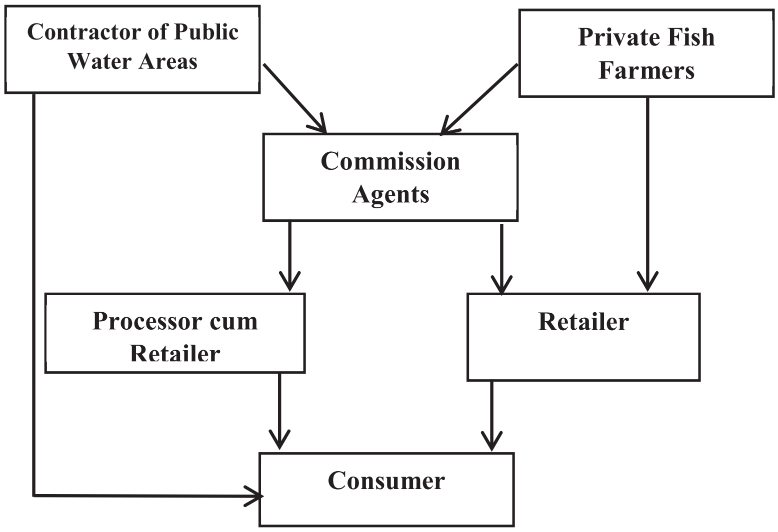


Fig. 1. Traditional Market Channel for Fish products, studied by SoyPak.

Market Intermediaries. Marketing margins are indicators of trends in costs, profits and services provided by farmers and food marketing firms. This is the difference between what the consumer pays for food and what the farmer receives (Kohl & Uhl, 1972). It is also calculated as the percentage share received by each marketing intermediary. There is a strong cumulative effect on the marketing margin resulting from the increasing number of intermediaries involved in marketing process (Rahman *et al.,* 2013; Bashir*etal,* 2001).

As shown in Fig. 1 above we can classify the whole marketing system into four major intermediaries involved in the flow of fresh water fish i.e., Contractor/fish farmers, (contractors typically have a license to fish or farm in public waters), Commission agents, retails, and processor cum retailer. Each intermediary has different variables which help in calculation of marketing margins.

1. **Contractor.** The contractors in the rural markets sell about 15 percent of the total catch while 85 percent is brought to the urban markets, which is sold through the commission agent. A contractor spends money on various activities involved from harvest to disposal of fish, these have been categorized below;

i)	Netting (average)	=	Rs. …. / 40 kg
ii)	Packing	=	Rs. …. / 40 kg
iii)	Transportation	=	Rs. …. / 40 kg

Cost of netting, packing and transportation vary per location of harvesting.

2. **Private Fish Farmers:** A major part of the total harvest from private fish farmers is sold in the urban market through the commission agents. Therefore the variable involved in the farmers marketing process is considerably different from that of contractor.

i)	Netting (average)	=	Rs. …. / 40 kg
ii)	Packing	=	Rs. …. / 40 kg
iii)	Transportation	=	Rs. …. / 40 kg
iv)	Labor	=	Rs. …. / 40 kg

Cost of netting, packing, Transportation and labor vary per location of harvesting.

3. **Commission Agents:** The commission agents (middleman = arhtiyas) operate in the town or city markets. They possess sizable cold storage facilities and are an important functionary in the marketing of freshwater fish. They grade the fish in accordance with the requirements of the buyers. Typically, a

commission agent has employed one or two laborers for handling, grading, storage and cleaning of fish and shop. The commission agent disposes of all the fish preferably on the same day or with the next few days. The commission agents receive 8-12 percent commission fees from the buyers of fish as is prescribed by the market committee. Commission agents also provide credit to producers and contractors with some conditions such as the producer or contractors will sell their produce through the commission agent and commission agent will charge a 7-10 percent commission fee from producer or contractors. Following items constitute cost structure for commission agents:

i)	Icing	=	Rs. …. / 40 kg
ii)	Labor charges	=	Rs. …. / 40 kg
iii)	Shop rent	=	Rs. …. / 40 kg
iv)	Miscellaneous	=	Rs. …. / 40 kg

Cost of icing, labor charges, shop rental, miscellaneous costs vary per location of outlets.

4. **Retailers.** There retailers purchase supplies from the commission agents or the contractors in the rural markets. Some of them sell fish to the urban consumers. Some have permanent shops in the urban markets generally close to the commission shops but a majority of them carry fish from place to place for sale to the final consumers. They are indirect contact with the consumers and supply various species and sizes of fish to the consumers according to their demand. Some retail outlets are able to cook fish and they sell both raw and cooked fish from the same source. Costs of the various variables involved in different activities are given below:

i)	Icing	=	Rs. …. / 40 kg
ii)	Packing	=	Rs. …. / 40 kg
iii)	Salt	=	Rs. …. / 40 kg
iv)	Labor charges	=	Rs. …. / 40 kg
v)	Miscellaneous	=	Rs. …. / 40 kg

Shopping Malls / super markets:

In recent years, the introduction of shopping malls / super markets in Pakistan has provided another platform for marketing of fish and is increasing day by day. These stores provide the benefit that fish is available through the year both iced and frozen (mostly imported fillets). Fig. 2 describes the fish market channel for the movement of fish from producer to shopping malls.

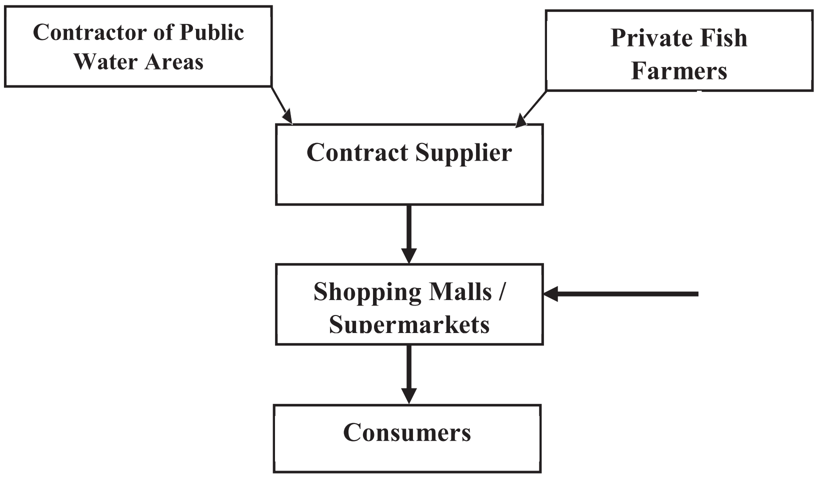


Fig. 2. Recently developed fish marketing channel through shopping malls in Pakistan. *Source:* SoyPak (ASA/WISHH)

Tilapia Market Development

Tilapia is native to Africa and it has been distributed all over the world. Now, it has become the second most common farmed group of fish species after the carps. The Genetically Improved Farmed Tilapia (GIFT) strain has been widely distributed after being developed by breeders in the Philippines and Malaysia. It is successfully cultured all over the world in both intensive and semi-intensive fish culture practices. Its preferred consumption size starts from 350 gram in some countries. It is hardy and can be cultured in high stocking densities up to 6,000 per acre, without water exchange or aeration. In more intensive culture, heavy feed inputs with additional aeration to meet its oxygen demands can triple production per acre. Due to its favorable characteristics it is gaining popularity both in culture and consumption in Pakistan.

Aquaculture Economics versus Dairy and Broiler Farming

Fish are cold blooded animals while cattle and broilers are warm blooded animals. Thus fish have low maintenance energy requirements but high protein demands. Fish tend to have much lower Feed Conversion Ratio (FCR) values than warm blooded animals. Relatively simple nutritional requirements, low FCRs and better feed consumption and absorption efficiencies increases profit margins and makes aquaculture a growing business of choice. The calculated return on investment (ROI) values and benefits cost ratios (BCR) for fish (tilapia), poultry (broiler) and cattle farming are 0.79,1.79,; 0.06,1.06 and 0.63,1.6respectively (Table 1). These values have been calculated on the basis of total recurring costs on a project. Capital cost along with their depreciation values have been avoided because the total economic value is calculated for one year only of the operation of the project.

Table 1. Comparison of ROI &BCR of tilapia, poultry (broiler) and cattle farming project

	ROI	BCR
Tilapia	0.79	1.79
Poultry	0.06	1.06
Cattle	0.63	1.6

Prepared by: SoyPak/ASA/WISHH and UVAS, Pattoki, Pakistan

RETURN ON INVESTMENT (ROI)

ROI measures the gain or loss generated on the investment relative to the amount of money invested. It is an indicator of the efficiency of an investment. The ROI enables us to compare one investment to another, informing an investor how profitable an investment will be relative to the amount of capital one has invested.

ROI Formulas:

$$\frac{(\text{Gain from Investment} - \text{Cost of Investment})}{\text{Cost of Investment}} = \text{ROI}$$

BENEFIT COST RATIO (BCR)

Benefit cost ratio is the ratio of the total value of the benefits to the cost. This method is more appropriate to determine the social and economic worth of a project. The benefit cost ratio can be estimated as follows:

Benefit Cost Ratio (BCR) = Net Benefit (NB)÷ Net Cost (NC)

ECONOMICS OF A TWO-ACRE TILAPIA FISH FARM

GIFT tilapia fry were imported from Thailand in the month of March 2013 and stocked in one two-acre pond. Fish were fed soy-based extruded floating feed which was manufactured locally on a recently installed extruder at Oryza Organic Pvt Ltd. (OOPL) with technical support of ASA/WISHH under FEEDing Pakistan Project funded by USDA. The fish were reared for a growth period of eight months and harvested in November and December 2013. Total recovery of fish after complete rearing period was 80 percent and average fish at harvest was 900 grams each. The total harvested fish was sold at the wholesale fish market Lahore.

ECONOMICS OF TWO-ACRE TILAPIA FARM

Table 2. Economics of Two-Acre Tilapia Fish Farm for the Year 2013

A-OPERATIONAL EXPENSES			
Items	**Unit**	**Cost /Unit (Rs.)**	**Total Cost (Rs.)**
Tilapia(GIFT) fry imported	11,700.00	8.00	93,600.00
Feed	10,867.00	65.00	706,355.00
Land lease	2.00	30,000.00	60,000.00
Water charges (Including electricity/diesel)	2.00	30,000.00	60,000.00
Labor charges	2.00	20,000.00	40,000.00
Aeration charges (electricity/diesel	2.00	10,000.00	20,000.00
Miscellaneous	2.00	5,000.00	10,000.00
Total Operational Expenses - Total operational expenses –A			989,955.00

B- INCOME		Sale/Unit (Rs.)	Total Sales (Rs.)
Total Biomass kg) (No. of saleable fish 9,360 at av. Wt. 0.9 kg)	8,424.00	210	1,769,040.00
Total –B			**1,769,040.00**
C-PROFIT			
Profit/Crop (B-A)			**779,085.00**
ROI			0.79
Benefit Cost Ratio (BCR) = (B/A)			1.79

Source: Department of Fishery and Aquaculture, UVAS-Pattoki, Pakistan

Table 3. Economics of 30,000 Broilers

A-OPERATIONAL EXPENSES			
Items	**Unit**	**Cost / Unit (Rs.)**	**Total Cost Rs.**
DOC price	30,000.00	30.00	900,000.00
Feed bags (3.5 kg/bird)	2,100.00	2,250.00	4,725,000.00
Vaccination & medication cost (Rs./ 8/bird)	30,000.00	8.00	240,000.00
Litter (Rs./ 2/bird)	30,000.00	3.00	90,000.00
Labor charges (Rs.4/bird)	30,000.00	4.00	120,000.00
Electricity and fuel charges (Rs. 12/bird)	30,000.00	12.00	360,000.00
Miscellaneous/unforeseen expenses (Rs. 2/bird)	30,000.00	2.00	60,000.00
Total operational expenses –A			6,495,000.00

B- INCOME			
Total live weight (kg), (No. of saleable birds with 3 % mortality, 29,682) assuming 1.8kg/bird	52,380.00	130.00	6,809,400.00
Sale of empty bags	2,100.00	8.00	16,800.00
Sale of old litter (trucks)			90,000.00
Total –B			6,916,200.00
C-PROFIT			
Profit/flock (B-A)			421,200.00
ROI			0.06
Benefit Cost Ratio (BCR) = (B/A)			1.06
Note: Prices of items can fluctuate			

Source: Department Of Poultry Production, UVAS-Pattoki, Pakistan

Table 4. Economics of Cattle Farm (52 Animals)

A-OPERATIONAL EXPENSES			
Items	**Unit**	**Cost/Unit (Rs.)**	**Total Cost (Rs.)**
Land Rent for 17 acre	17.00	35,000.00	595,000.00
Feed Cost (Concentrate)	1.00	1,525,590.00	1,525,590.00
Feed Cost (Green Fodder + Wheat Straw)	1.00	2,150,000.00	2,150,000.00
Labor (4 person x 12 months = 48)	48.00	10,000.00	480,000.00
Health care & breeding	52.00	9,327.00	485,004.00
Mortality (adults @ 2% [one animal])	1.00	150,000.00	150,000.00
Mortality (adults @ 5% [three claves)	3.00	50,000.00	150,000.00
Electricity and fuel per month	12.00	20,000.00	240,000.00
Miscellaneous/Unforeseen expenses (@ Rs.10,000/month)	12.00	12,000.00	144,000.00
Total Operational Expenses – A			5,919,594.00

B-INCOME		
Items	**Particulars**	**Total Sales (Rs.)**
Buffalo milk	22x3,000 liter. @ Rs. 60/liter	3,960,000
Cow milk	22x3,000 liter @ Rs. 50/liter	3,300,000
Animals sold	Culled cows = 3 @ Rs. 100,000	300,000
	Culled Buff = 3 @ Rs. 100,000	300,000
	Male young stock = 20 @ Rs. 50,000	1,000,000
	Female young stock = 10 @ Rs. 70,000	700,000
Skins	4 (1 large @ Rs, 1000, 3 small @ Rs. 500)	2,500
Farm Yard Manure	20 trolleys @ Rs. 5000 per trolley	100,000
Total –B		9,662,500.00
C-PROFIT		
Profit/flock (B-A)		3,742,906
ROI		0.63
Benefit Cost Ratio (BCR) = (B/A)		1.6
Note: Prices of items can fluctuate		

Source: Department of Livestock Production, UVAS-Pattoki, Pakistan

TILAPIA

Globally, tilapia culture ranges from rural subsistence farming to large-scale commercial operations depending on intensity of management employed. The use of different culture systems (earthen pond, concrete tank, raceway, or cage) and management strategy (extensive, semi-intensive, intensive, monoculture, polyculture, mono-sex culture, and mixed sex culture) depends on the farmer's resources, site characteristics, environmental conditions, socio-economic factors, technological know-how and market demand. Production costs and yields vary from country to country depending on the level of management used.

The last three decades have seen significant developments in farming of tilapias worldwide. Tilapias are not only the second most important farmed fish globally, next to carps, but have also been described as likely the most important aquaculture species of the 21st century (Fitzsimmons, 2000). The fish is being farmed in 140 countries worldwide with about 98% of tilapia produced outside their original habitats (FAO, 2013). The production of farmed tilapia is among the fastest expanding food sectors in the world. Nile tilapia (*Oreochromis niloticus*) is the most cultured fresh water species among the farmed tilapia and contributes about71% of the world total tilapia production (FAO, 2013).

Genetically Improved Farmed Tilapia (GIFT) mono-sex tilapia is a new phenomenon of commercial production since 2012 in Pakistan. The FEEDing Pakistan Project took the lead to bring tangible success stories of commercial GIFT tilapia production system in Pakistan and entitled it ***"The Blue Revolution"*** in Pakistan.

Pakistan's Fisheries Development Board (FDB) imported about 50,000 GIFT Tilapia from Thailand in the year 2011. The tilapia fry exhibited a high rate of early mortality and there was no significant success due to lack of both quality floating feed and technical knowhow. From 2012, under FEEDing Pakistan Project, the technical support and trainings to stakeholders were provided for better farm management; usage of quality floating feed and improvement in transportation helped in reducing the early mortality.

From 2011 to 2014, total imported tilapia fry were 3.680 million and total locally sex-reversed tilapia fry were 1.7 million by Tawakkal Tilapia Hatchery in Muzaffargarh, Punjab – Pakistan (Table 5).

Table 5. Total imported tilapia fry from Thailand and total locally sex-reversed tilapia fry.

Year of Import	Import of GIFT tilapia fry from Thailand	Major areas of farming
2011	50,000	Thatta, Murdkee, Managla
2012	330,000	Bhawal Nagar, Pattoki, Alipur Chatta, Muzaffargarh, Sheikupura, Thatta, Mangla
2013	1,600,000	Bhawal Nagar, Pattoki, Gujranwala Muzaffargarh, Sheikupura, Thatta, Mangla, Rajan Pur, Rangpur, Jhang, Sarghoda
2014	1,700,000 (And also 1,700,000 locally produced)	Bhawalpur, Bhawal Nagar, Pattoki, Gujranwala Muzaffargarh, Sheikupura, Thatta, Mangla, Rajan Pur, Rangpur, Khadal, Jhang, Thatta, Sajawal

Source: FDB, SoyPak (ASA/WISHH) and Tawakkal Tilapia Hatchery

In early 2012

FEEDing Pakistan program of ASA/WISHH in collaboration with Kansas State University and with local partner SoyPak, funded by USDA, started working to promote the aquaculture sector in Pakistan from October 2011. After an assessment of Pakistan's aquaculture sector by Prof. Dr. Kevin Fitzsimmons, University of Arizona, U.S.A., Dr. John Woiwode, Principal Scientist with AquaMatrix, U.S.A., and a former three year resident of Pakistan working on carp hatcheries with Punjab Fisheries Department, and Dr. R.S.N. Janjua, SoyPak, one of their recommendations was to conduct demonstrations of soy-based floating feed and introduction of mono-sex GIFT Tilapia with improved management supervision in Pakistan.

Under FEEDing Pakistan a memorandum of understanding (MOU) was signed between ASA/WISHH and the Fisheries Development Board (FDB). Due to the lack of high quality local fry, FDB arranged importation of tilapia fry from Thailand and ASA/WISHH provided 44,000 lbs. of floating feed made with U.S. soybean meal to partner farmers. The project also hired recent fishery graduates as Field Research Officers (FROs) to work in the field with the farmers. Dr. Janjua took the lead to hold demonstrations under the lead technical supervision of Prof. Dr. Fitzsimmons. There was high early mortality during the nursing stage in imported Tilapia probably due to long hauling distances from country of origin to the destination farms and changing environmental conditions. The high mortality contributed to higher costs, along with overhead expenses, slightly discouraging the fish farmers to adopt GIFT Tilapia culture practices. These concerns were overcome through education including farmers' field days, seminars and publication of 2012 Tilapia Feeding Demonstration, conferences and training under the FEEDing Pakistan Project.

Tilapia Feeding Demonstrations in late 2012

The 2012 tilapia feeding demonstrations were conducted utilizing soy-based floating fish feed at seven sites throughout Pakistan (refer to map), of which three were floating cages and the remaining four were ponds. FDB handed over tilapia fingerlings to ASA/WISHH with body weights ranging from 10 g to 25 g to stock at seven demonstration sites listed in Table 6. The trials were showcases for soy-based

extruded floating feed to demonstrate the revolutionary increase in production per acre with improved growth and better FCR Table 6.

- Tilapia were monitored for growth while utilizing extruded, pelletized floating fish feed produced with U.S. soybean meal (SBM) and compared with locally produced sinking compressed pellet feed with domestic ingredients.
- The efficacy of the soy-based fish feed was tested in both ponds and floating cages in local conditions in Punjab and Sindh—the main aquaculture areas in Pakistan.

Results from the feeding demonstrations showed that the tilapia grew to sizes not often seen with traditional feeding methods; the time required from placement of fry to harvest was also reduced.

Table 6. Feeding Demonstration Final Results

Feeding Demonstration Final Results:					
#	**Farm Site**	**# of fish harvested**	**Avg. size of fish (g)**	**Final biomass (kg)**	**FCR**
Earthen Ponds Culture					
1	Himalaya, Muredkhe,	1,600	770	1,232	1.18
2	Chatta, Ali Pur Chatta	4,406	596	2,626	1.96
3	Ejaz Maqbool, Thatta	2,838	460	1,536	1.69
4	Khonbhati, Thatta	2,991	480	1,445	2.20
Floating Cages Culture					
5	Salli Dam (cage culture)	849	501	425	2.55
6	Keenjhar Lake (cage culture)	3,447	663	2,286	1.37
7	Mangla Dam (cage culture	4,728	610	2,880	1.53

Source: ASA/WISHH/SoyPak

The demonstrations yielded the following results:

- FCRs ranged from 1.18 to 2.55 with an average of 1.74
- Yield varied from 1.2 tons/acre to 7.3 tons/acre

The main purpose of conducting trials in 2012 was to demonstrate the better FCR and improved yield per acre that could be achieved using soy-based floating feed and best management practices. The harvest at each site was conducted by holding a Farmers' Field Day (FFD) where first time stakeholders largely farmers, traders, provincial Department of Fisheries, FDB, Marine Fisheries department, academia, students and selected media witnessed the harvest of larger size tilapia ranging from 460 g to 770 g (Table 6).

In 2013

The successful demonstration in 2012 gave encouragement to farmers to enter in the tilapia business and investors to start a feed manufacturing business in the following year of 2013. 2013 can be declared as the first year of tilapia farming on commercial scale in Pakistan. That year, around 1.6 million tilapia fry were imported from Thailand and farmed mostly in the southern region of Punjab.

Under Feeding Pakistan, two milestones were achieved in bringing" The Blue Revolution" in the aquaculture sector of Pakistan with technical and financial support of ASA/WISHH. First was installation of a state of the art extruder, E-750 with capacity of five ton per hour made by Extru-Tech Inc. USA, to establish the first production plant of soy-based extruded floating fish feed by Oryza Organic Pvt. Ltd. (OOPL) located in a suburb of Lahore. Second, the first tilapia hatchery in the private sector was constructed by Tawakkal Hatchery Management at Muzaffargarh. Under an MOU with OOPL, ASA/WISHH also provided a container load of U.S. Hi-Pro SBM 25 MT to produce trial feeds along with technical guidance by Dr. Chhorn Lim, senior aquaculture nutrition and feed development scientist at the Aquatic Animal Health Research Unit of the U.S. Department of Agriculture (USDA), Agriculture Research Service (ARS, Auburn, Alabama, U.S.A.).

There were three sites selected for the trials with locally produced soy-based extruded floating feed produced by OOPL on a trial basis and imported tilapia by fish farmers. Mostly the trials were focused on the effects of stocking density of GIFT Tilapia on growth and FCR in different regions. Employees at one site, Tawakkal Fish Farms, learned record keeping and it was decided to conduct a case study of the economic benefits of using powdered on-farm mixed feed (Chapter V) vs. soy-based floating feed.

Case Study 2013

Economic benefit of using soy-based floating feed versus powdered home-mixed feed (mash) and impact on GIFT tilapia growth and yield per acre in Muzaffargarh

Introduction

In 2013, Tawakkal Fish Farm, located in the fish farming cluster around Muzaffargarh, imported tilapia fry from Thailand and stocked them in 10 ponds of different sizes. The farmer had experience producing and using powdered feed in carp culture and had decided to use powdered feed which costs 2 times less than floating feed.

Methodologies

Site Selection:

District Muzaffargarh has a large cluster of fish farms encompassing an area of nearly 6,000 acres because of favorable weather conditions and availability of both ground water and river water.

However, a high risk of seasonal flood in monsoon periods is always there.

One of the progressive farmers accepted this challenge and trials were started. Required records were properly maintained. After several discussions with the farmer, it was decided to allocate one pond to soy-based floating feed and one pond to powdered feed. All the records were maintained and the same treatment of feeding and pond management was practiced. The stocking density of 5,000 tilapia per acre was maintained in both ponds. Tilapia fry were stocked in mid-March 2013 and first harvest was carried out on November 07, 2013 by holding a farmers' field day. The economics of tilapia production is calculated in Table 7 and Table 8 on the basis of Operational Expenses.

Picture 1. Famers Field Day at Tawakkal Fish Farm (TFF), Muzzafargarh, Dated November 07, 2014 (Photo courtesy: R.S.N. Janjua, SoyPak.)

L-R: Mr. Zafar, Director, TFF, Mr. Zahid Yaqoub, C.E.O. OOPL, Mr. Javed Iqbal, Director-TFF, Mr. M. Ashraf, Director General, Punjab Fisheries Department, Mr. Shahid Iqbal, Director-TFF, R.S.N. Janjua, Country Rep. ASA/WISHH (Photo courtesy: R.S.N. Janjua, SoyPak)

Picture 2. Farmers Field Day at Tawakkal Fish Farm - Muzaffargarh (November 07, 2014) (Photo Courtesy: R.S.N. Janjua, SoyPak)

Picture 3. L-R: Mr. Khawar Awan, Director Inland Fisheries, Sindh Fisheries Department, R.S.N Janjua, Countery Rep. ASA/WISHH, Mr. Ghulam Mohammad Mahar, Director General, Sindh Fisheries Department & Trainee at Tilapia Production, Chilia - Thatta in 2013.

Table 7. Economics of Tilapia Culture on Powdered Feed

Pond 9 Density: Tilapia 5,000 per acre			
A-OPERATIONAL EXPENSES			
Items	**Unit**	**Cost /Unit (Rs.)**	**Total Cost (Rs.)**
Tilapia fry for nursery (0.2 gm)	6,000.00	8	48,000.00
Powdered mash (FCR 3.83)	10,344.00	27.00	279,288.00
DAP (Bag 50 kg)	2.00	3,600.00	7,200.00
Urea (Bag 50 kg)	2.00	1,700.00	3,400.00
Cow-dung/poultry waste	1.00	6,000.00	6,000.00
Land Lease	1.00	13,000.00	13,000.00
Water charges (Including electricity/diesel)	1.00	25,000.00	25,000.00
Labor charges	1.00	16,000.00	16,000.00
Miscellaneous	1.00	15,000.00	15,000.00
Total Operational Expenses - Total operational expenses – A			412,888.00
B-RETURN		**Sale/Unit (Rs.)**	
Tilapia: Total Biomass kg) (No. of saleable fish 5000 at Avewt.0.54 kg)	2,700.00	180	486,000.00
Total –B			486,000.00
C-PROFIT			
Profit/Crop (B-A)			73,112.00
ROI			0.15
Benefit Cost Ratio (BCR) = (B/A)			1.17

Table 8. Economics of Tilapia Culture on Soy-based Floating Feed

Pond-4 Density: Tilapia 5,000 per acre			
A-OPERATIONAL EXPENSES			
Items	**Unit**	**Cost /Unit (Rs.)**	**Total Cost (Rs.)**
Tilapia fry for nursery (0.2 g)	6,000.00	8.00	48,000.00
Extruded floating feed for tilapia (FCR 1.00:1.23)	4,256.00	70.00	297,920.00
DAP (Bag 50 kg)	2.00	3,600.00	7,200.00
Urea (Bag 50 kg)	2.00	1,700.00	3,400.00
Land lease	1.00	13,000.00	13,000.00
Water charges (Including electricity/diesel)	1.00	25,000.00	25,000.00
Labor charges	1.00	16,000.00	16,000.00
Miscellaneous	1.00	15,000.00	20,000.00
Total Operational Expenses - Total operational expenses -A			430,520.00
B-RETURN		**Sale/Unit (Rs.)**	
Tilapia: Total Biomass kg (No. of saleable fish = 5000 at ave.wt.0.690kg)	3,450.00	20.000	690,000.00
Total –B	3,450.00		690,000.00
C-PROFIT			
Profit/Crop (B-A)			259,480.00
ROI			0.60
Benefit Cost Ratio (BCR) = (B/A)			1.60

Conclusion:

The major contribution in cost of production is feed. In Pond 9, the cost of powdered feed was 66.5 % of total cost of production and in Pond 4 the cost of soy-based floating feed was 69.2% of total cost of production (Table 7 and Table 8respectively). The profit per acre was 3.9 times higher in Pond 4 (Table 8) as compared to Pond 9 (Table 7). Table 9 shows that the ROI and BCR were higher in Pond 4, the pond using soy-based floating fish feed as compared to Pond 9 which used powdered feed.

Table 9. Comparison of Economics and Profit per acres Tilapia growth on soy-based floating feed vs powdered feed

Table Numbers	**Table 8 (Pond 4)**	**Table 7 (Pond 9)**
Stocking Detail (No. of Fish/acre)	5,000	5,000
Date of Stocking (avg. wt. 2 g) imported from Thailand	Mar-14	Mar-14
Date of first Harvesting	November 07,2013	November 07,2013
No. of days	221	221
Expenses (Rs)	430,520.00	412,888.00
Revenue (Rs)	690,000.00	486,000.00
Av. Wt. at first harvesting (kg)	0.69	0.54
Biomass (kg)	3,450.00	2,700.00
Cost per kilogram	124.79	155.51
Profit per acre	259,480.00	73,112.00
Return on Investment (ROI)	0.60	0.15
Benefit Cost Ratio (BCR) = (B/A)	1.60	1.17

The cost of production of fish was Rs. 124.79 per kg in the soy-based extruded floating feed pond (Table 8 Pond 4) which was comparatively lower than the cost of production per kg (Rs. 155.51) in the powdered feed pond (Table 7 Pond 9). The case study shows that productivity and profit per acre on soy-based floating feed is higher than using powdered feed.

In 2014

These results enticed additional farmers to shift from conventional fish farming to semi-intensive tilapia culture. Provision of low cost tilapia seed as compared to imported tilapia fry and quality soy-based floating feed attracted the attention of these progressive fish farmers. The tilapia hatchery started commercial production of sex reversed tilapia fry in early 2014.

Pictures . L-R: R.S.N. Janjua, Mr. Javed Iqbal and Mr. Shahid Iqbal, Directors, First Private Sector Tawakkal Tilapia Hatchery, Muzaffargarh, Punjab. Mr. Shahid displaying female tilapia as mouth brooder with clutch of eggs in mouth. (Photo Courtesy: R.S.N. Janjua, SoyPak (ASA/WISHH)

Case Study 2014

Simple Economics of varying densities of GIFT tilapia when polycultured with rohu/thaila on a combined feeding schedule in Rangpur- Muzaffargarh District. Punjab-Pakistan

Introduction

In 2014, M/s Shahzad-Ijaz fish farm located in Ranjpur, a tehsil of district Muzaffargarh was selected for the first commercial study on polyculture of GIFT *Oreochromis niloticus* with Indian major carps, *Catla catla* (thaila) and *Labeo rohita* (rohu). The locally developed sex-reversed GIFT tilapia was purchased from Tawakkal Tilapia Hatchery in Muzaffargarh. The purpose of running trials was to provide a showcase of tilapia production as a new profitable business entity in the cluster of major Indian carp fish farming region i.e., Rangpur in Pakistan. Traditionally, farmers stock rohu or thaila at an average rate of 800 per acre in Rangpur. By introducing high density of tilapia stocking (mono-culture) and combinations of tilapia and rohu/thaila (polyculture), the additional income to farmers with the same available resources of water and land and use of soy-based extruded floating feed in tilapia production can increase fish yields as compared to traditional fish farming.

In continuation of trials and demonstrations since 2012 and these innovations, this case study of polyculture with tilapia intervention has been accomplished successfully. The details of stocking densities, combination rations, quality and quantity of feed offered in trials and total production along with ROI and BCR has been provided in the following paragraphs.

Methodologies

Site Selection:

Tehsil Rangpur, District Muzaffargarh is a big cluster of farms encompassing of area of approximately 2,000 acres. This area is unique due to its quality water both for fish farming and agriculture and favorable weather characteristics. Prior to the start of the trial, this area was thoroughly surveyed and various farmers were interviewed to accept the challenge of polyculture with GIFT tilapia. One of the progressive farmers accepted this challenge and trials were started. Required records were properly maintained.

Observation

The findings of the trials in terms of fish production per unit and economics have been tabulated and summarized in the Table 10 besides other details have been discussed in the proceeding paragraphs.

Table 10. Summary of economics of four different trials with varying fish stocking densities of rohu/thaila and GIFT tilapia (locally sex-revered tilapia)

Table #	Stocking (No. of fish / acre)	Expenses (Rs)	Revenue (Rs)	Production (kg)	Cost per kg	Profit per acre	ROI	BCR
Table 11	Rohu, Thaila 700	252,937.50	415,800.00	1,540.00	164.25	162,862.50	0.64	1.64
Table 12	Rohu, Thaila 720 + Tilapia 650	267,835.00	482,870.00	2,018.00	137.18	206.035.00	0.74	1.74
Table 13	Tilapia 3,000 + Rohu, Thaila 800	380,750.00	738,801.00	3,304.80	115.21	358,051.00	0.94	1.94
Table 14	Tilapia 7,200	444,911.20	890,848.00	4,345.60	102.38	445,936.80	1.00	2.00

Total production in Trial 1 (Table 11) was 1,540 kg, in Trial 2 (Table 12) 2,018 kg, in Trial 3 (Table13) 3,304.80kg and in Trial 4 (Table14) 4,345.60kg. In Trial 1 only two species, rohu (90%) and thaila (10%) were grown, in Trial 2 in addition to 700 rohu and thaila, 650 tilapia were also stocked, in Trial 3 tilapia was the major species (3,000) while 800 hundred rohu and thaila were also stocked. Trial 4 was monoculture of tilapia only.

When production per unit was compared among all the trials, highest production per acre was attained in monoculture tilapia (Table14) while the lowest was obtained from polyculture of rohu and thaila (Table11). Total production was highest in Trial 4 (Table 14) where only tilapia was stocked in the fish pond. Similarly all the other economic parameters like ROI and BCR were also highest in Trial 4 (Table 14).

Picture: Case Study 2014 Harvesting Polyculture (Rohu / Thaila and Tilapia before a large group of farmers in Rangpur, district Muzaffargarh. (Photo courtesy R.S.N. Janjua, SoyPak (ASA)

The study reveals that monoculture of GIFT tilapia is more profitable than all the other different culture options tested in these trials. The possible reasons for better production are: i) better conversion of feed and ii) better resistance against poor water quality parameters. In addition tilapia has a short production period as compared to conventional semi-intensive culture of Indian and Chinese major carps. From the trials, the positive point is that its marketable size can be achieved with the stocking of much small sized fish tilapia than in the case of carps. Other than monoculture (tilapia), its polyculture with rohu and thaila also gave reasonable production per acre and was ranked at second position (Table13) during these trials. So from these observations, it can be concluded that monoculture of tilapia is the best option. But combination of these three species (GIFT tilapia, rohu and thaila) can also be adopted comfortably in existing ponds. The use of supplementary soy-based extruded floating feed gave much higher production; see Table 14,when compared with Table 11 and 12.

Table 11.Economics of Poly-Culture: Indian Carps (rohu & thaila)

Pond 2 density: Rohu and Thaila = 700 per acre			
A-OPERATIONAL EXPENSES			
Items	**Unit**	**Cost /Unit (Rs.)**	**Total Cost (Rs.)**
Rohu, thaila @ 200 gram each advanced fingerling	700.00	35.00	28,000.00
Powdered mash for rohu (FCR 3.75)	5,775.00	22.50	129,937.50
DAP (Bag 50 kg)	2.00	3,600.00	7,200.00
Urea (Bag 50 kg)	2.00	1,700.00	3,400.00
Cow-dung/poultry waste	1.00	6,000.00	6,000.00
Land Lease	1.00	13,000.00	13,000.00
Water charges (Including electricity/diesel)	1.00	25,000.00	25,000.00
Labor charges	1.00	16,000.00	16,000.00
Miscellaneous	1.00	24,400.00	24,400.00
Total Operational Expenses - Total operational expenses - A			252,937.50
B-RETURN		**Sale/Unit (Rs.)**	
Rohu, thaila: Total biomass kg) (No. of saleable fish 700 at avg. wt. 2.2 kg)	1,540.00	270.00	415,800.00
Total –B			415,800.00
C-PROFIT			
Profit/Crop (B-A)			162,862.50
ROI			0.64
Benefit Cost Ration (BCR) = (B/A)			1.64

Source: Combined Study of SoyPak and Tawakkal Hatchery 2014

Table 12. Economics of Polyculture: Indian Carps (rohu & thaila) & tilapia

Pond 1 density: Rohu and thaila = 720 per acre and tilapia = 650 per acre			
A-OPERATIONAL EXPENSES			
Items	**Unit**	**Cost / Unit (Rs.)**	**Total Cost (Rs.)**
Rohu, thaila @ 200 gram each	700.00	35.00	24,500.00
Tilapia Seed for nursery (0.2 g)	650.00	3.00	1,950.00
Extruded Floating Feed for tilapia (FCR 1.00)	618.00	70.00	43,260.00
Powdered mash for rohu (FCR 3.75)	5,250.00	22.50	118,125.00
DAP (Bag 50 kg)	2.00	3,600.00	7,200.00
Urea (Bag 50 kg)	2.00	1,700.00	3,400.00
Land lease	1.00	13,000.00	13,000.00
Water charges (Including electricity/diesel)	1.00	25,000.00	25,000.00
Labor charges	1.00	16,000.00	16,000.00
Miscellaneous	1.00	24,400.00	24,400.00
Total Operational Expenses -Total operational expenses -A			276,835.00
B-RETURN		**Sale/Unit (Rs.)**	
Tilapia: Total biomass kg (No. of saleable fish 618 avg. Wt. 1.0 kg)	618.00	215.00	132,870.00
Rohu, thaila: Total biomass kg) (No. of saleable fish 700 at avg. wt. 2.0 kg)	1,400.00	250.00	350,000.00
Total –B	2,018.00		482,870.00
C-PROFIT			
Profit/Crop (B-A)			206,035.00
ROI			0.74
Benefit Cost Ration (BCR) = (B/A)			1.74

Source: Combined Study of SoyPak and Tawakkal Hatchery 2014

Table 13. Economics of Poly-Culture: Indian Carps (rohu & thaila) & tilapia

Pond 7 density: Rohu, thaila = 800 per acre and tilapia = 3,000 per acre			
A-OPERATIONAL EXPENSES			
Items	**Unit**	**Cost /Unit (Rs.)**	**Total Cost (Rs.)**
Fingerling Rohu, Thaila @ 200 gram each	800.00	35	28,000.00
Tilapia Seed for nursery (0.2 g)	3,000.00	3.00	9,000.00
Extruded Floating Feed for tilapia (FCR 1.00)	2,025.00	70.00	141,750.00
Powdered mash for rohu (FCR 3.75)	4,800.00	22.50	108,000.00
DAP (Bag 50 kg)	2.00	3,600.00	7,200.00
Urea (Bag 50 kg)	2.00	1,700.00	3,400.00
Land lease	1.00	13,000.00	13,000.00
Water charges (Including electricity/diesel)	1.00	25,000.00	30,000.00
Labor charges	1.00	16,000.00	16,000.00
Miscellaneous	1.00	24,400.00	24,400.00
Total Operational Expenses -Total operational expenses -A			380,750.00
B-RETURN		**Sale/Unit (Rs.)**	
Tilapia: Total biomass kg (No. of saleable fish 2,700 avg. wt. 0.750 kg)	2,025.00	210.00	425,250.00
Rohu, Thaila: Total biomass kg) (No. of saleable fish 790 at avg. wt. 1.62 kg)	1,279.80	245.00	313,551.00
Total –B	3,304.80		738,801.00
C-PROFIT			
Profit/Crop (B-A)			358,051.00
ROI			0.94
Benefit Cost Ration (BCR) = (B/A)			1.94

Source: Combined Study of SoyPak and Tawakkal Hatchery 2014

Table 14. Economics of monoculture: Tilapia

Pond 5 density: Tilapia = 4,500 per 0.625 acre = 7,200 tilapia per acre			
A-OPERATIONAL EXPENSES			
Items	**Unit**	**Cost /Unit (Rs.)**	**Total Cost (Rs.)**
Tilapia Seed for nursery (0.2 g) 4,500 per .625 acre & converted to stocking density per acres = 7,200 fry	7,200.00	3.00	21,600.00
Extruded Floating Feed for Tilapia (FCR 1)	4,780.16	70.00	360,500.00
DAP (Bag 50 kg)	1.00	3,600.00	3,600.00
Urea (Bag 50 kg)	1.00	1,700.00	1,700.00
Land lease	1.00	13,000.00	13,000.00
Water charges (Including electricity/diesel)	1.00	25,000.00	30,000.00
Labor charges	1.00	16,000.00	16,000.00
Miscellaneous	1.00	24,400.00	24,400.00
Total Operational Expenses -Total operational expenses -A			444,911.20
B-RETURN		Sale/Unit (Rs.)	
Tilapia: Total Biomass kg (No. of saleable fish 6,400 at avg. wt. 0.679 kg)	4,345.60	205.00	890, 848.00
Total –B			890,848.00
C-PROFIT			
Profit/Crop (B-A)			445,936.80
ROI			1.00
Benefit Cost Ration (BCR) = (B/A)			2.00

Source: Combined Study of SoyPak and Tawakkal Hatchery 2014

Conclusion

This was the first formal study of monoculture and polyculture with tilapia in Pakistan. There were several challenges on the ground including communication in remote area. Year 2014 is the third year of commercial tilapia production and demand for large tilapia over 600 g is fetching good prices, which encourages the progressive farmers to switch over to tilapia culture. The case study shows the potential of polyculture with tilapia for additional income and high ROI and BCR (Table 15). The study also observes there was no report of in-breeding of locally sex-reversed tilapia.

Picture: Case Study 2014 Harvesting Tilapia on Farmers Field Day in Rangpur, district Muzaffargarh. Photo courtesy R.S.N. Janjua, SoyPak (ASA/WISHH)

Table 15. Comparison of economics and density in monoculture and polyculture in Pakistan

Table Numbers	**Table 11 (Pond 2)**	**Table 12 (Pond 1)**	**Table 13 (Pond 7)**	**Table 14 (Pond 5)**
Stocking Detail (No. of Fishes/Acre)	Rohu, thaila 700	Rohu, thaila 720 + tilapia 650	Tilapia 3,000 + rohu, thaila 800	Tilapia 7,200
Date of Stocking (Avg. Wt. in grams)	01APR2014 (200 gram)	01APR2014 (Rohu + thaila 200 g), 28AUG2014 (Tilapia 200g)	01APR2014 (Rohu + thaila 200g), 28AUG2014 Tilapia 200g)	21APR2014 (Tilapia 0.2g)
Date of first Harvesting	15DEC2014	15DEC2014	15DEC2014	15DEC2014
No. of days	239	239	239	239
Harvested (No. of fish/acre)	Rohu/thaila 700	Rohu, thaila 700 + tilapia 618	Tilapia 2,700 + rohu, thaila 790	Tilapia 6,400
Avg. Wt. (kg)	Rohu/thaila 2.2	Rohu/thaila: 2.0 & tilapia: 1.0	Rohu/thaila: 1.62 & tilapia: 0.750	tilapia: 0.679
Total Calculated Biomass (Production in kg)	1,540.00	2,018.00	3,304.80	4,345.60
Cost Per Kilogram	164.25	137.18	115.21	102.38
Expenses (Rs)	252,937.50	267,835.00	380,750.00	444,911.20
Revenue (Rs)	415,800.00	482,870.00	738,801.00	890,848.00
Profit Per Acre	162,862.50	206,035.00	358,051.00	445,936.80
Return on Investment (ROI)	0.64	0.74	0.94	1.00
Benefit Cost Ration (BCR)	1.64	1.74	1.94	2.00

Source: Evaluation of case study by R.S.N. Janjua, SoyPak (ASA/WISHH) and Tawakkal Tilapia Hatchery, Pakistan

Conclusion

Most of the fish produced were sold on site by the farmers. Accurate data on Pakistan's inland fish population, production, and marketing and disease incidences should be collected and considered with this data. It is difficult for small holder farmers owning one or two small farm units to organize their marketing. Thus fish farmers should be encouraged to organize into cooperatives, associations, or public groups to facilitate inputs to increase production and improve quality as well as to market their surplus produce at advantageous prices. Lack of proper marketing and non-accessibility to market results in an environment for farmers to be exploited by buyers in various ways. The prevailing marketing system is dominated by commission agents and needs capacity building of all stakeholders to develop a marketing network and marketing information system. In this regard the private sector can play a vital role to strengthen the market network.

References

Ashraf, M., M.J. Khan and M.A. Saleem, 1988.*Marketing of fish in Punjab*. Punjab Economic Research Institute, Lahore.

Government of Pakistan, 2013.Economic Survey of Pakistan, Finance Division, Economic Advisor's Wing, Islamabad.

Chhorn Lim, Carl D. Webster, 2004, Tilapia, Biology, Culture, and Nutrition.

FAO 2013. Fisheries and Aquaculture Department: Global Aquaculture Production Statistics

Fitzsimmons, K. 2000. Tilapia: the most important aquaculture species of the 21st century. **In:** Proceedings from the Fifth International Symposium on Tilapia Aquaculture (pp. 3-8). Rio de Janeiro.

Infofish International 2012. The Fisheries and Aquaculture sector of Pakistan.

Kohl, R.L. and J.N. Uhl, 1972. Marketing of Agricultural Products. 5th Ed., Macmillan Publishing Co., Inc. New York.

Rahman, M. M., Hossain, M. A., Tasnoova, S., Ahamed, F., Hossain, M. Y., & Ohtomi, J. 2013. Fresh Fish Marketing Status in the Northwestern Bangladesh: Recommendations for Sustainable Management. Our Nature, 10(1), 128-136.

Shepherd, W.A., 1996. A guide to marketing costs and how to calculate them. Marketing and Rural Finance Service, Agricultural Services Division. FAO. Rome.